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Reprinted from BARTONIA, No. 19, issued March 8, 1938

New Stations for Lophotocarpus spongiosus in Southern New Jersey

J. W. ADAMS

The recent explorations by Dr. John M. Fogg, Jr., and Mr. Bayard Long along the Delaware River have shed light on the distribution of a number of native species once considered rare in southern New Jersey and in one case unknown. Two outstanding examples are *Lilaeopsis chinensis* (L.) Kuntze and *Lophotocarpus spongiosus* (Engelm.) J. G. Sm.

Since its first discovery along the Delaware River, *Lilaeopsis chinensis*[1] has been found at so many stations and in such abundance that now it is considered common along the brackish tidal marshes.

In 1936 Dr. Fogg records the first substantiated addition of *Lophotocarpus spongiosus*[2] to the flora of southern New Jersey. This present note places on record two more of what may eventually become a series of stations paralleling those of *Lilaeopsis chinensis* in frequency.

On June 17, 1937 the Torrey Botanical Club made a field trip to study the tidal vegetation along the Delaware in Salem County. The writer while riding in the company of Dr. Fogg, who acted as guide, and Miss Flora S. Fender noticed along the exposed tidal mud, 2.5 miles southwest of Salem, plants suggesting *Lophotocarpus*. Since it was decided best not to stop at the time, the return trip was made by the same route. Examination proved the plants to be a colony of *Lophotocarpus spongiosus* in all its leaf-shapes and plant sizes. While most of the plants were in full bloom, a great number still had quite young flower-buds tightly nestling among the leaves; other plants were young and immature: these indicated that the colony had from days to weeks more of anthesis.

There was a conspicuous absence of other more fully developed plants, there being only a few clumps of *Peltandra virginica* and young plants of *Ranunculus sceleratus* growing nearby. On the mud at the bases of some of the *Lophotocarpus* were tangled mats of *Spirodela polyrhiza* abandoned by the receded tide.

Between the time the above mentioned station was observed and our return to it Dr. Fogg and the party found another locality which had as fine a display of the *Lophotocarpus* as could be expected anywhere. On an extensive tidal mud flat, behind the shore of the Delaware River at Elsinboro Point, 4 miles southwest of Salem, were countless plants in bloom. It is safe to say there were acres of them.

The plants in general appeared to be more fully developed than those of the previous locality, both in size of the plants and in their stage of bloom. This

[1] Bartonia 16: 51. 1934.
[2] Bartonia 17: 21. 1935.

species dominated that particular mud flat at the time. As before, the two associated plants were *Peltandra virginica* and *Pontederia cordata* with *Baccharis halimifolia* along the margin.

One seeing such an impressive abundance of a plant, once unknown to southern New Jersey, cannot help but wonder, as suggested in the first paragraph, how much more of it is yet undiscovered. It also brings to mind the fact that while concentrated effort was being made to explore the Pine Barrens by local (and distant) botanists, Salem and Cumberland counties, and especially along the Delaware, seem to have been neglected. Anyone at all within the area of this plant, from the middle of June until frost, could not possibly have overlooked such an abundantly conspicuous display of it.

Specimens of *Lophotocarpus spongiosus* from both stations were collected and distributed to the following herbaria: *J. W. Adams* No. 3309, University of Pennsylvania, Philadelphia Botanical Club, Gray Herbarium, University of Montreal, Dr. Walo Koch in Zurich, Switzerland; *J. W. Adams* No. 3296, Gray Herbarium, University of Montreal, Dr. Walo Koch; *John M. Fogg, Jr.* No. 11,890, University of Pennsylvania, Philadelphia Botanical Club.

MORRIS ARBORETUM OF THE UNIVERSITY OF PENNSYLVANIA

Reprinted from BARTONIA, No. 19, issued March 8, 1938

Populus heterophylla in Southern New Jersey

J. W. ADAMS

One of the rarest trees in southern New Jersey is the Swamp Cottonwood or Downy Poplar, *Populus heterophylla* L., which is common along borders of river swamps in the southern states and the Mississippi valley. Along the Atlantic States it extends from Connecticut to Georgia, being rare and local in the northern portions of this range. In New Jersey this species has been listed in Mr. Norman Taylor's Flora of the Vicinity of New York [1] as occurring in "Bergen, Hudson, Middlesex and Cumberland counties, not common; also at Cape May Court House."

Dr. Witmer Stone in his Plants of Southern New Jersey [2] says "known only from one tree, found by Mr. Albert Commons, July 27, 1880, on Fortesque Beach." He has seen a specimen in the Herbarium at New Brunswick labeled "low woods on Delaware Bay, June 27, 1880" which forms a basis for his record and apparently for the citation in Dr. N. L. Britton's Catalogue of the Plants found in New Jersey.[3]

Until 1911 the Fortesque Beach station was the only one known for southern New Jersey. On August 13, 1911, Mr. Bayard Long discovered a second station along the Coast Strip bordering the brackish marshes below Cape May Court House, Cape May County.[4] No others have been found since.

A third station was found on June 21, 1935 by the writer while riding with his wife and Dr. John M. Fogg, Jr. to collect along the Delaware River at Sea Breeze. As we passed along the road cutting through the swampy woods and thickets, about 2 miles east-southeast of Lanings Wharf, Cumberland County, several trees were noticed which had a none-too-familiar look. After we had finished collecting along the river we returned by the same route and stopped to examine the trees carefully. They turned out to be the *Populus heterophylla* for which local botanists had been seeking additional stations for some time.

The present station is a comparatively short distance from Mr. Commons' locality. There is every reason to believe that more stations for this species will be found as botanists collect in this little-explored region of the state.

The trees at Lanings Wharf locality were about 20–25 feet tall, forming a small grove in the open margin of the swampy woods. At the time the roots were under several inches of water. That the water at the spot was permanent was evidenced by the fact that within the shadow of the trees was a fairly broad pool, about a foot or so deep, in which grew *Potamogeton* sp. with entirely submerged and floating leaves, while almost under water, near the margin, were colonies of *Proserpinaca palustris*. The other nearby trees and shrubs, typical of southern New Jersey deciduous swamps, were mainly *Acer rubrum* var. *tridens*, *Magnolia virginiana*, *Ilex verticillata*, etc.

PAGINATION BEGINS

Herbarium specimens in duplicate were collected as well as living root-sprouts for the Morris Arboretum tree collections. At the time of writing the sprouts have grown over 4 feet tall. The herbarium specimens cited, together with their distribution, are as follows: *John M. Fogg, Jr.*, No. 8800, University of Pennsylvania, Gray Herbarium; No. 8801, University of Pennsylvania, University of Missouri, Field Museum; *J. W. and Myrtle T. Adams*, No. 2037, Morris Arboretum, Philadelphia Botanical Club, Arnold Arboretum, U. S. National Herbarium.

MORRIS ARBORETUM OF THE UNIVERSITY OF PENNSYLVANIA

[1] Mem. New York Bot. Gard. 5: 262. 1915.
[2] Ann. Rep. New Jersey State Museum for 1910: 391. 1911.
[3] Geol. Surv. New Jersey 2: 227. 1889.
[4] Bartonia 4: 16. 1911.

Reprinted from PLANT PHYSIOLOGY, 12: 455–470, 1937.

EXCHANGE OF ELECTROLYTES BETWEEN ROOTS AND ACID SOLUTIONS[1]

RUTH BEALL

(WITH FIVE FIGURES)

Introduction

This paper presents, in abbreviated form, some results of a study of changes in electrical conductance and in hydrogen-ion concentration of dilute acid solutions exposed to the action of seedling roots of *Lupinus albus* L. Effects of these solutions on the plants are also described.

Materials and methods

Three inorganic acids (HCl, H_2SO_4, and HNO_3) and three organic acids (formic, acetic, and propionic) were tested separately, each at three concentrations, 0.00002 N, 0.00008 N, and 0.00016 N. Expressed as molar fractions these concentration values are M/25,000, M/12,500, and M/6250 for HCl, HNO_3, and the organic acids; and they are M/50,000, M/25,000, and M/12,500 for H_2SO_4. The water used was redistilled from Pyrex glass and had a conductance of approximately 2 millionths reciprocal ohm. For control cultures DOAK's (**11**) modification of HARTWELL and PEMBER's nutrient solution was used, as well as H_2O.

Seeds of white lupine were germinated in finely chopped sphagnum and the resulting seedlings were introduced into the test solutions when primary roots and hypocotyls were about 6 cm. and 3 cm. long, the cotyledons being still within the seed coats. After rinsing, four seedlings were introduced into each culture jar. The jars used were 500-ml. Pyrex beakers, each containing 500 ml. of liquid. The seedlings were supported by means of cotton in holes in the jar lids, which were perforated Petri-dish covers of Pyrex glass (**2**). The roots were wholly submerged and the hypocotyls were entirely above the liquid surface. Thus nearly the same area of root surface was exposed to the solution in every case.

These experiments were carried out in a dark-room at the Botanical Laboratory of the University of Pennsylvania. For each set of solutions there were two series of experiments, the temperature for one series being about 22° C. for the mineral acids, and about 23° C. for the organic acids; that for the other series being about 28° C. Temperature fluctuated somewhat in each case, within plus or minus 2°.

[1] This experimentation was carried out with the financial aid of a Lydia T. Morris Fellowship of the Morris Arboretum of the University of Pennsylvania.

IRREGULAR PAGINATION

Changes in ionic concentration of the solutions surrounding the roots were followed by means of daily measurements of electrical resistance, from which were derived the corresponding specific conductance values, computed for a temperature of 18° C. Daily measurements of hydrogen-ion concentration were made by means of the quinhydrone-titration method, with McIlvaine's buffers (**4**). Since the temperature coefficient of hydrogen-ion concentration is so small as to be negligible in such a study as this (**7**), the pH values were not corrected for temperature. Daily observations concerning the appearance of the plants were also made, particularly with regard to development and visible injury.

In the following subsections, changes in the media will be presented first, distilled water and the several acid solutions being considered in order, and then attenion will be turned to records of growth and of injury to the plants.

Experimentation

Changes in the media

Distilled water.—Figure 1 presents graphically average daily con-

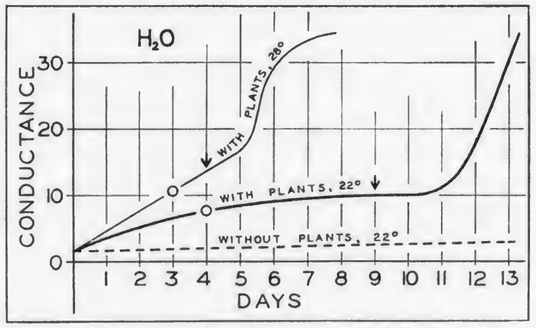

Fig. 1. Conductance (millionths recip. ohm) of H_2O with and without seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

ductance values for H_2O with and without seedlings at 22° C., and for H_2O with seedlings at 28° C. Since no considerable conductance increase occurred without plants, it may be inferred that the increases shown by water with plants were due to outward movement of material from the roots. There was continuous exosmosis of electrolytes, in excess of any absorption that may have occurred. Net exosmosis, which was at first relatively slow, became markedly accelerated about the fifth day at the higher temperature and about the eleventh day at the lower temperature. It was at all times more rapid at the higher temperature than at the lower. At the lower temperature the pH value of H_2O with seedlings increased from 5.4 to 5.7 in

the first day, and then to 6.1 in the following three days, after which it remained practically unchanged. At the higher temperature the changes were very similar, but more rapid, from 5.0 to 5.5 in one day, and to 6.1 in three days. This may indicate either that there was some absorption of hydrogen ion, the effect of which on conductance was overbalanced by relatively greater exosmosis of electrolytes, or that exosmosis acted to reduce the hydrogen-ion concentration of the surrounding liquid.

Mineral acid solutions.—The manner in which conductance of the mineral acid solutions in contact with seedling roots first decreased and subsequently increased is shown by the graphs of figures 2 to 4. Each graph

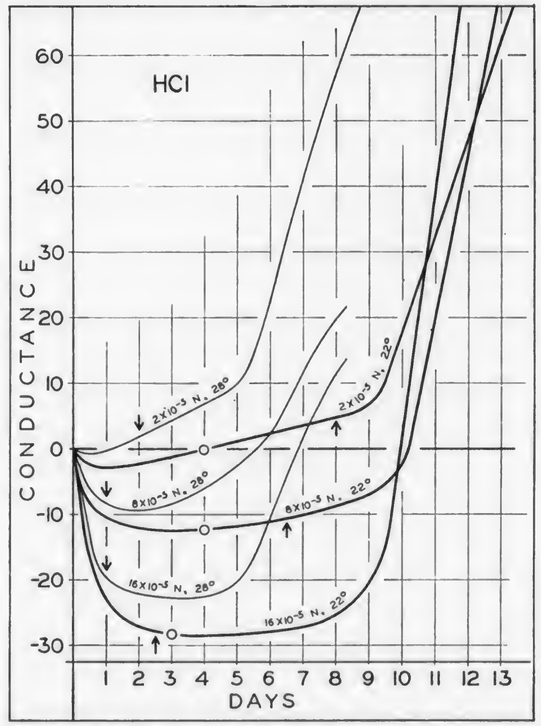

Fig. 2. Conductance change (millionths recip. ohm) in HCl solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

starts at zero on the scale of ordinates (which, in each case, represents the initial conductance of the solution in question) and conductance decreases are plotted as negative while increases are plotted as positive. The three average initial conductances, expressed as reciprocal ohms $\times 10^{-6}$, for each of the three concentrations of each acid were as follows: HCl, 8.9, 26.8, 52.8; H_2SO_4, 8.8, 26.9, 56.2; HNO_3, 8.5, 31.3, 64.5.

Some special measurements made at the end of the first hour showed, in many instances, a slight initial increase in conductance, relatively greater as the original concentration of the solution was higher, which suggests that there may have been an immediate exosmosis of electrolytes from the roots. This detail is not shown on the graphs; it was not seriously studied and warrants no more than mention here.

Each of the graphs shows an initial decrease in conductance, which was very slight and of very short duration for the solutions of lowest concentration, but progressively more pronounced and of longer duration as concentration was higher. Considering the two graphs for each concentration together, it may be said that the period of conductance decrease was shortest

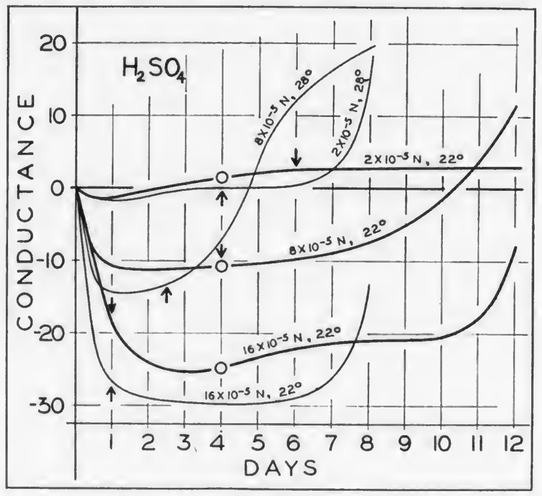

FIG. 3. Conductance change (millionths recip. ohm) in H_2SO_4 solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

(about 1 day or less) for the lowest concentration, longer (about 2 to 3 days) for the intermediate concentration, and longest (about 3 to 4 days) for the highest concentration. Similarly, the average amounts of conductance decrease were approximately as follows, the unit being 1×10^{-6} reciprocal ohm: for the lowest concentration, 2 units (all three acids); for the intermediate concentration, 11 units (HCl), 13 units (H_2SO_4), and 17 units (HNO_3); for the highest concentration, 26 units (HCl), 28 units (H_2SO_4), and 41 units (HNO_3). It is notable that HNO_3 showed greater conductance decrease at the two higher concentrations than was shown by either of the other mineral acids at these concentrations.

As to the temperature relations of the amount of conductance decrease, the available data permit no satisfactory generalization for all three mineral acids. From the graphs of figure 2 it is clear that the amount of conductance decrease for solutions of HCl was regularly somewhat greater at the lower than at the higher temperature. For the lowest and highest concentrations of HNO_3 (fig. 4) the same temperature relation apparently holds, but for the intermediate concentration of HNO_3 and for all tested concen-

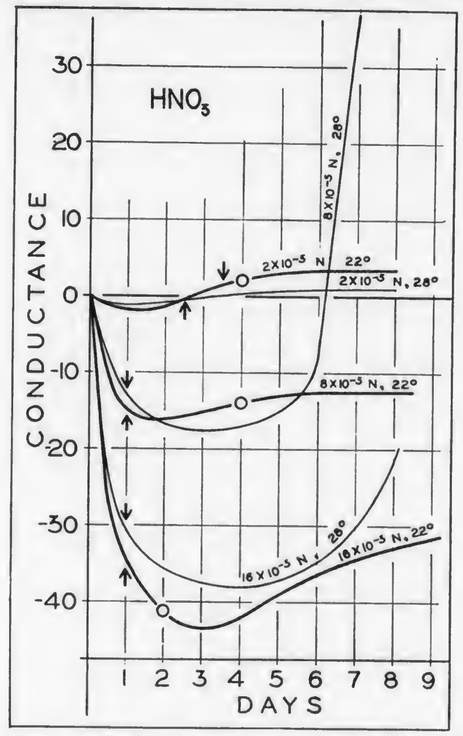

FIG. 4. Conductance change (millionths recip. ohm) in HNO_3 solutions with seedlings. Circles mark end of rapid initial increase in pH; arrows mark onset of visible root injury.

trations of H_2SO_4 (fig. 3) conductance decrease was more or less markedly greater at the higher than at the lower temperature.

When conductance decrease was relatively large in amount its rate is seen to have been rapid at first and then progressively more gradual. For the intermediate and highest concentrations the initial period of decrease was followed by a more or less prolonged period during which conductance was nearly maintained. This period was longer for the lower temperature

than for the higher one. After conductance began to increase, its rate of change sometimes remained low to the end of the experimental period, but in many cases (notably all the graphs of figure 2) its rate was soon rapidly accelerated, so that the final portions of the graphs ascend very steeply.

As to changes in hydrogen-ion concentration, the pH values of all tested solutions—and that of H_2O also—increased from the beginning of the experimental period onward. This increase was most rapid during the first day and became progressively slower till a value of about 6.0 was reached, when hydrogen-ion equilibrium with the roots was apparently established. Eventually the solutions became slightly more basic. The initial pH values of the solutions, corresponding to the initial conductances given above, were as follows: HCl, 4.6, 3.9, 3.6; H_2SO_4, 4.5, 3.9, 3.6; and HNO_3, 4.5, 3.8, 3.5. After one day the following values were reached: HCl, 4.8, 4.6, 5.3; H_2SO_4, 5.1, 4.6, 5.5; and HNO_3, 4.9, 5.1, 5.6. The time required, at the lower temperature, for each medium to attain a pH value of about 6.0 is shown by the position of the circle on the corresponding graph of figures 1 to 4. That time was in general somewhat shorter for the solutions of highest concentration than for the other solutions and for H_2O. The two lower concentrations of the three acids were essentially alike, reaching this value in four days; for the highest concentration the change was somewhat more rapid in HNO_3 (2 days) than in HCl (3 days) or H_2SO_4 (4 days).

Organic acid solutions.—Conductance changes shown by the three organic acids in contact with seedlings are set forth by the graphs of figure 5, which are plotted like those of figures 2 to 4. For each of the three concentrations of each acid, the initial conductance (each plotted as zero on the ordinate scale) was as follows: HCOOH, 6.2, 16.2, 29.3; CH_3COOH, 5.5, 10.6, 16.3; C_2H_5COOH, 4.2, 9.6, 14.1. For each solution the two temperatures tested showed no essential differences in conductance change; the graphs of figure 5 represent the average values for the two temperatures. As in the case of the mineral acids, conductance measurements made within the first day of the experimental period indicated in some instances a very slight immediate increase in conductance, of very short duration, which is not shown on the graphs.

These organic acid solutions showed an initial decrease in conductance, similar to that shown by the mineral acid solutions, but less pronounced. For the lowest concentration of each acid this decrease was of one day's duration or less. It was scarcely noticeable in the case of the most dilute solution of propionic acid, only very slight in the weakest acetic acid solution, and somewhat more pronounced in the lowest concentration of formic acid. In all these cases conductance subsequently increased gradually for about three days, after which it remained approximately unchanged. For

solutions of higher concentration the initial conductance decrease lasted somewhat longer, from one to three days, after which there was, in most cases, some increase, but this was never sufficient to raise the conductance of the solution above its original value. In general, conductance remained unchanged after five days. As was shown for the inorganic acid solutions, initial decrease was greater in amount as solution concentration was greater, the amounts of decrease (in conductance units) being as follows: HCOOH, 2, 11, 20; CH_3COOH, 1, 4, 8; C_2H_5COOH, 0.1, 3, 6.

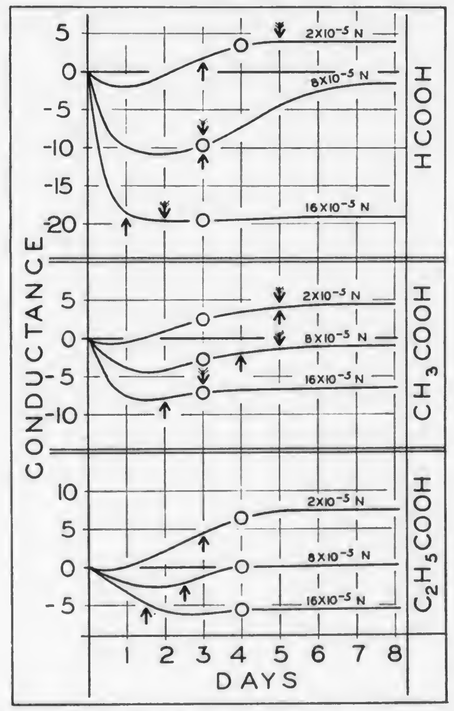

Fig. 5. Conductance change (millionths recip. ohm) in organic acid solutions. Circles mark end of rapid initial increase in pH; winged arrows mark onset of visible root injury at 23° C., naked arrows at 28° C.

Only the graphs for the lowest concentration of acetic acid and of propionic acid and the ones for the lowest and intermediate concentrations of formic acid show any considerable conductance increase within the 8-day period, but of course the conductance of the other organic acid solutions might have increased eventually had the period been longer. To make possible satisfactory comparisons among all six acids, with reference to con-

ductance increase, it would apparently have been necessary to employ experimental periods longer than 8 days.

Turning to the changes in hydrogen-ion concentration of the organic acid solutions, these were very nearly like the corresponding changes that occurred in the mineral acid solutions, except that the original solutions were generally less acid and the amount of change was correspondingly less. The initial pH values of the three concentrations of each of the three organic acids were as follows: HCOOH, 4.5, 3.9, 3.6; CH_3COOH, 4.8, 4.5, 4.1; and C_2H_5COOH, 4.9, 4.5, 4.2. In general there was rapid decrease in acidity during the first day, the following pH values being reached: HCOOH, 5.6, 5.7, 5.8; CH_3COOH, 5.5, 5.3, 5.3; and C_2H_5COOH, 5.3, 4.5, 4.3. Subsequently the decrease in acidity continued, but was more gradual, the solutions attaining a value of about 6.0 by the end of 3 days (intermediate and highest concentration of HCOOH and all three concentrations of CH_3COOH) or at the end of 4 days (lowest concentration of HCOOH and all concentrations of C_2H_5COOH), after which pH was approximately maintained. These times are indicated by the positions of the circles on the graphs of figure 5.

Physiological effects

Controls.—On the roots of the control plants, in nutrient solution, lateral branches always appeared within the first day of the experiment and these subsequently increased in number and enlarged as the primary root continued to elongate. On the second day, when the hypocotyl was well extended, the cotyledons diverged, and later the plumule opened and elongation of the epicotyl began. No chemical injury was apparent and these plants appeared to be quite healthy throughout the entire experimental period of 14 days, excepting that, being in darkness or very weak light, they were markedly etiolated.

Plants with roots in water.—Seedlings with roots in H_2O developed more slowly than those with roots in nutrient solution. Development of lateral roots was similar to that in nutrient solution except that these did not appear until after 2 days and they did not elongate so much. After 2 days also the cotyledons began to diverge and gradually development of the epicotyl proceeded. Injury at the tips of the roots was evident after about 9 days at the lower temperature and after about 4 days at the higher temperature, as is indicated by the arrows of figure 1. Injury to hypocotyls followed in many cases.

Plants with roots in mineral acid solutions.—In solutions of mineral acids development was still more retarded than in water and injury was apparent earlier. Injury was first noticeable as a slight constriction in the primary root, 1 to 2 mm. from the tip. The tip region soon became

flaccid and translucent, then very soft and brown. These changes gradually spread upward until, in the most severe cases, only the upper centimeter or less of the root was apparently free from necrosis. Injury to the hypocotyl usually followed soon after the onset of root injury. It was first evident as a translucent region 1 to 2 cm. long, about the middle of the hypocotyl, and necrosis spread both upward and downward. Subsequently the injured region of the hypocotyl turned brown and collapsed, sometimes with exudation, whereupon the cotyledons and plumules drooped. The plumule developed only slightly or not at all.

Development in the mineral acid solutions was roughly in inverse relation to the original concentration of the solution. Neither primary root nor hypocotyl elongated to any considerable extent in the solutions of highest concentration and both showed less elongation in the solutions of intermediate concentration than in the most dilute solutions. Lateral roots appeared after 2 days in all cases; in solutions of lowest concentration they developed throughout practically the entire length of the primary root, in solutions of intermediate concentration they were confined to the upper two-thirds of the main root, and in solutions of highest concentration they were confined to the upper one-third or less. When the region of their development was restricted they were unusually crowded. At the end of the experimental period they were longer in all mineral acid concentrations than in water, and their length was greater as the region supporting them was shorter. At either temperature, injury to the roots became evident earlier and progressed more rapidly as solution concentration was higher, and for any tested concentration it appeared earlier and progressed more rapidly at the higher than at the lower temperature. At the end of the experimental period injury in the least concentrated solutions was confined to the terminal region of the primary root, in solutions of intermediate concentration it occupied from one-third to one-half of the length of the main root, and in solutions of highest concentration the primary root was visibly injured throughout practically its entire length. Injury to the hypocotyls also appeared earlier and progressed more rapidly as the solution was more concentrated and as the temperature was higher. If the times required for the onset of root injury in these solutions (as shown by the positions of the arrows in figures 2 to 4) are considered roughly as reciprocally proportional to the toxicity of the solutions, then it appears that, for the two lower concentrations at the lower temperature, the decreasing order of toxicity for these three mineral acids was HNO_3, H_2SO_4, HCl. For the highest concentration at this temperature HNO_3 and H_2SO_4 were about alike and produced injury sooner than HCl. For the lowest and for the intermediate concentration at the higher temperature HNO_3 and HCl were about alike and produced injury more promptly than H_2SO_4. Finally, all three acids were approximately alike, on the basis of this time criterion, for the

highest concentration at the higher temperature since all had produced injury within the period before the first examination, but in these cases injury was noticeably more severe in HNO_3 solutions than in the other acids. It thus appears that both temperature and concentration took part —along with the chemical characteristics of the acids—in determining the promptness of the onset of injury.

PLANTS IN ORGANIC ACID SOLUTIONS.—Development was more rapid and more extensive in the solutions of organic acids than in the corresponding solutions of mineral acids. With formic and acetic acids development was somewhat greater in the lowest concentrations than in the others; with propionic, however, development in the solution of intermediate concentration equalled or exceeded that in the most dilute solution, and development in the most concentrated solution was less rapid than in either of the other solutions. By the second day lateral roots had emerged in all cases. They appeared earlier, however (after only 1 day), in the most concentrated solutions of formic acid and acetic acid at the higher temperature, and in all solutions of propionic acid at this temperature. Like the mineral acids tested, the organic acids permitted the development of laterals throughout most of the length of the primary root in solutions of lowest concentration. As the solution was more concentrated laterals were increasingly restricted to the older region of the primary root, but the region bearing laterals was not so limited as under the corresponding influence of the mineral acids. As with the inorganic acid solutions, but to a lesser extent, lateral roots were finally more closely crowded and longer the more restricted was the region from which they arose.

Injury by the organic acids was similar, in inception and nature, to that produced by the mineral acids, but for any tested concentration and temperature it generally appeared somewhat later with the most rapidly injurious organic acid than with the least rapidly injurious mineral acid, as is shown by comparing the positions of the arrows on the graphs of figures 2 to 5. (It should be noted that in figure 5 winged arrows refer to the lower temperature while naked arrows refer to the higher temperature.) Acetic acid generally appears to have been less promptly injurious than either of the other organic acids, which were apparently about alike in this respect. In general, injury by any solution was less prompt at the lower than at the higher temperature and it appeared sooner as the concentration was higher.

THE SIX ACIDS AND H_2O COMPARED AS TO TOXICITY

Although the available data are inadequate for a satisfactory comparative study of the degrees of toxicity of all six acids, it may be of interest to summarize the lengths of time required for the onset of visible root injury, as follows:

1. At 22° or 23° C. the descending order of indicated toxicity for each concentration is:
   a. For lowest concentration: HNO_3 (3.5 days), HCOOH and CH_3COOH (5 days), H_2SO_4 (6 days), HCl (8 days).
   b. For intermediate concentration: HNO_3 (1 day), HCOOH (3 days), H_2SO_4 (4 days), CH_3COOH (5 days), HCl (6.5 days).
   c. For highest concentration: HNO_3 and H_2SO_4 (1 day), HCOOH (2 days), HCl (2.5 days), CH_3COOH (3 days).
2. At 28° C. the descending order of indicated toxicity for each concentration is:
   d. For lowest concentration: HCl (2 days), HNO_3 (2.5 days), HCOOH and C_2H_5COOH (3 days), H_2SO_4 (4 days), CH_3COOH (5 days).
   e. For intermediate concentration: HNO_3 and HCl (1 day), H_2SO_4 and C_2H_5COOH (2.5 days), HCOOH (3 days), CH_3COOH (4 days).
   f. For highest concentration: HNO_3, H_2SO_4, HCl and HCOOH (1 day), C_2H_5COOH (1.5 days), CH_3COOH (2 days).

For each combination of temperature and concentration the reciprocal of the injury-time for each acid may perhaps be taken as an index of its relative toxicity. If for each combination all six reciprocals are expressed in terms of the reciprocal for HNO_3 and if the resulting relative toxicity values for each acid are averaged, the final outcome is a single series of tentative toxicity indices that may represent all the tested combinations of temperature and concentration; that new series is as follows: HNO_3, 1.00; HCl, 0.71; H_2SO_4, 0.66; C_2H_5COOH, 0.63; HCOOH, 0.61; CH_3COOH, 0.41. The corresponding value for H_2O is 0.22. According to these averages, it appears on the whole: (a) that HNO_3 was the most toxic, while (b) CH_3COOH was least toxic of the six acids considered, (c) the former being about 2.5 times as toxic as the latter; (d) that HCl and H_2SO_4 were nearly alike in toxicity (about 69 per cent. as toxic as HNO_3); (e) that C_2H_5COOH and HCOOH were nearly alike in toxicity (about 62 per cent. as toxic as HNO_3), (f) only slightly less toxic than HCl and H_2SO_4, and (g) about 50 per cent. more toxic than CH_3COOH; and, finally, (h) that H_2O appeared to be about half as toxic as CH_3COOH and about one-fifth as toxic as HNO_3. While these statements seem to agree in general with some conclusions that are suggested by superficial scrutiny of the graphs and their appended arrows, it must be remembered that they are intended only to represent a sort of summary of the toxicity phase of the present study, for it is almost certain that the degrees of toxicity of these acids in any test depend on temperature and concentration as well as on chemical nature.

Conclusion

The initial reduction in conductance of these acid solutions was evidently due to absorption of ions by the roots. This absorption was probably accompanied by some exosmosis in all cases, as was shown for H_2O.

While it is conceivable that the conductance of a solution might be diminished by exosmosis alone, if the materials extruded from the roots were of such nature as to remove from solution the ions present, consideration of the nature of the solutions examined in this study and of the kinds of material that might possibly escape from plant roots leads to the conclusion that the observed conductance decrease must have been due to direct removal of ions by the plants. This was no doubt accomplished principally by absorption of material into the cells, but adsorption of ions on to root surfaces may have occurred also. Since solutions of all the acids tested were reduced in conductance, and hence in ion concentration, it seems safe to conclude that ions were absorbed from both inorganic and organic acid solutions.

The amount of net absorption from the several solutions seems to have varied directly with ion supply. For any acid, the higher the ionic concentration of the original solution, the greater and more rapid was the removal of electrolytes. And among the six acids tested, the greater the degree of ionization, in general, the more rapid was net absorption. A general index of absorption from these acid solutions under all the conditions represented by these experiments may be derived by expressing the average amount of net absorption from each concentration of each acid in terms of the average net absorption from the corresponding concentration of HNO_3, and averaging the three values for each acid. This index is as follows: HNO_3, 1.0; H_2SO_4, 0.83; HCl, 0.77; HCOOH, 0.73; CH_3COOH, 0.30; and C_2H_5COOH, 0.12. That is, considering the average of the results of the several experiments, there was greatest absorption from HNO_3 solutions, and least from those of C_2H_5COOH. Net absorption from the organic acid solutions was generally less than from the corresponding concentrations of mineral acids, but HCOOH was only slightly below HCl and H_2SO_4 in this respect. This is in general agreement with the order of dissociation of these acids.

Absorption was, however, apparently influenced by other factors to some extent. Examining the results for the two temperatures separately it may be seen that, for the several mineral acids at the lower temperature, net absorption from HNO_3 solutions was greatest and that from H_2SO_4 solutions was least, net absorption from HCl being intermediate. This order of penetration ($HNO_3 > HCl > H_2SO_4$) is in agreement with DAVIDSON and WHERRY'S (**9**) results with wheat seedlings. On the other hand, in the present study the order of penetration at the higher temperature is: $HNO_3 > H_2SO_4 > HCl$; this is the order reported by HIND (**15**) from her experiments with potato tuber. Net absorption was apparently increased by rise in temperature in the case of all solutions of H_2SO_4 and the intermediate concentration of HNO_3, but in the case of the other solutions (namely, all concentrations of HCl and the lowest and highest concentrations of HNO_3) net absorption was apparently decreased. DELF (**10**) and SKEEN (**20**) found the permeability of plant tissues to increase with rise in temperature, and STILES and JØRGENSEN (**24**) report greatly augmented absorption of hydrogen ion under similar conditions. ECKERSON (**13**), on the other hand, reports that the effect of rise in temperature on permeability of root cells varied according to the species considered. Studies on this subject reported in the literature have been carried out with a variety of materials and by means of a variety of experimental procedures. It is safe to presume that rates of penetration of materials into plant roots, and rates of absorption, depend on the kind and condition of the roots as well as on temperature and the nature of the solutions from which the absorbed substances are derived.

The pronounced decrease in hydrogen-ion concentration which accompanied the initial decrease in conductance of these solutions may be taken as evidence that there was rapid absorption of hydrogen ion in the early part of each experimental period. As was the case with absorption in general, this was always greater the higher the concentration of the original solution, and greater the first day than in any succeeding day. Absorption of hydrogen ion by plant cells has been reported by many observers (**5, 14, 18, 19, 23**).

The toxicity of the hydrogen ion has long been recognized. About forty years ago KAHLENBERG and TRUE (**17**) pointed out that the toxic influence exerted upon plants by dilute solutions of strong acids was probably largely due to this ion. Since that time BREAZEALE and LECLERC (**3**), HOAGLAND (**16**), and DUNN (**12**), among others, have reported on acid toxicity for plant tissues. As to the effect of such toxicity within plant cells, ADDOMS (**1**) observed that the protoplasm of root hairs injured by an acid medium appeared to have been coagulated.

It seems probable that the toxic effects produced by the acid solutions of the present study were due chiefly to the hydrogen ion. Similar injuries appeared in all experimental plants and this was the only ion, other than the hydroxyl ion, which was common to all solutions. Also the hydrogen ion is more active, and probably more toxic, than any of the anions present.

In general, degree of toxicity followed hydrogen-ion concentration. For any acid the higher the concentration and the greater the ionic absorption therefrom, the more promptly did injury occur, and in the acids as a group it was generally true that the more completely dissociated the acid the more toxic it was. Propionic acid was, however, more toxic than would be expected from its ionic concentration or from the amount of ionic absorption that occurred from its solutions. Apparently the propionic molecule also was toxic. This is in agreement with TRUE'S (**25**) idea that the harmful action of formic and acetic acids is due principally to hydrogen ion, whereas that of propionic acid is due chiefly to the undissociated molecule. Other

workers also have attributed the toxicity of organic acid solutions to undissociated molecules (**6, 8, 12**). Even in the case of the acids whose toxicity seems to have been fundamentally due to excess of hydrogen ion, other ions or molecules may not have been without influence on toxicity.

The toxicity of these acids was generally greater at the higher temperature than at the lower. Injury was apparent earlier and the plants became more severely affected at the higher temperature. SKEEN (**21**) also has found toxicity to be increased by increase in temperature in some cases.

Although, as has been intimated, it appears likely that the initial decrease in conductance of the acid solutions was fundamentally due to hydrogen-ion absorption by the roots, there is indirect evidence that other ions and molecules also were absorbed. Furthermore there seems to be no reason to doubt that there was in all cases some exosmosis of ions or molecules, or both. That the initial lowering of solution conductance ceased—generally after 1 to 3 days for the mineral acids, and after a somewhat shorter time for the organic acids—must have been due to decreased absorption, to increased exosmosis, or to both together. Either of these alterations in the rate of movement of material across the root periphery may well have been occasioned by increasing toxic action. Indeed this increased exosmosis has been used as a criterion of injury by some workers (**8, 22**). With the mineral acids excessive exosmosis from the poisoned plants eventually became greatly pronounced, as was shown by increase in solution conductance, but no rapid outburst of electrolytes had occurred at the time the experiments with the organic acids were discontinued. Nor did rapid exosmosis occur within 8 days in solutions of aromatic acids tested in a similar way by MOTHER CHRYSOSTOM (**8**).

In those cases where conductance increased rapidly toward the end of the experimental period, the sudden increase in exosmosis that is indicated may have been due to a corresponding sudden increase in outward permeability of the root cells to electrolytes, such as might result from some sorts of poisoning, or perhaps poisoning may first have caused general protoplasmic coagulation, followed at length by a sudden disintegration. It is possible that the anions or undissociated molecules of the organic acids acted in some way to retard the poisoning process or hinder protoplasmic disintegration.

Summary

1. Roots of *Lupinus albus* were found to absorb electrolytes from very dilute solutions of certain inorganic acids—HCl, H_2SO_4, HNO_3—and certain organic acids—HCOOH, CH_3COOH, C_2H_5COOH. Absorption was greater in amount and more rapid the higher the original concentration of the solution. For any tested concentration it was greater as dissociation was more nearly complete.

2. There was apparently a rapid absorption of hydrogen ion, probably accompanied by some absorption of other ions, of molecules, or of both.

3. Injury to the plants resulted in all cases. This is probably to be attributed to absorbed hydrogen ion in each instance except that of propionic acid, whose undissociated molecules seem to have been largely responsible for the injury.

4. In many cases rapid exosmosis of materials from the roots eventually occurred, indicating increased permeability of cell membranes or rapid disintegration of cell contents.

5. Influence of temperature, solution concentration, and hydrogen-ion concentration on absorption and exosmosis and on growth and development of the seedlings are among the topics discussed.

This problem was suggested by Professor RODNEY H. TRUE, Director of the Morris Arboretum, who maintained a continuous interest throughout the study. In the analysis of the results and in the preparation of this paper the writer received many helpful suggestions from Dr. BURTON E. LIVINGSTON, of the Johns Hopkins University, and from Dr. CHARLES A. SHULL, of the University of Chicago.

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A NEW FOSSIL OAK WOOD FROM IDAHO[1]

Irwin Boeshore and J. Austin Jump

Although several hundred species of fossil oaks from the United States have been described from their leaf impressions, comparatively little work has been done on the fossil wood of the genus. This neglect may be attributed in part to the uncertainty of specific differentiation on the basis of characters of the wood anatomy, since positive identification of oak species often cannot be made from wood structure alone. However, the sub-genera of the genus *Quercus* may be determined by the microscopic examination of their wood anatomy, and many of their included species also may be identified by this means. Thus it seems reasonable that well preserved specimens of fossil wood might be expected to show similar distinctions.

Most of the work on fossil oak wood has been done by German investigators who described a number of western American species as well as European ones. This work is so little known that Trelease (1924), in monographing the oaks of America, stated that the only fossil oak wood to receive a specific name was *Quercus Marcyana* Pen., although at that time more than a dozen species had been described. However, the descriptions of these oaks might have been overlooked very easily since they were not published in widely distributed journals.

The first description of fossil oak wood was published in 1839 by Göppert, who described fossil wood from Silesia and northern Germany and named it *Klödenia quercoides*. At that time he mentioned the nearly complete agreement of *K. quercoides* and *Quercus pedunculata* and six years later changed the generic name to *Quercites*, the genus in which fossil leaves of oak are now placed. However, Unger in 1842 had proposed the genus *Quercinium* for fossil oak wood and had pointed out that probably *Klödenia* Göpp. should be changed to *Quercinium*. Very little notice was taken of the genus until Felix (1883, 1884) published the descriptions of the species known at that time and added four new ones. In the first of these papers he showed that the three species previously described by Unger were based on invalid characters and therewith included them in the group of species upon which he published. Penhallow (1891) described a fossil wood from the post-glacial of Illinois that strongly resembled the modern chestnut oaks and named it *Quercus Marcyana*, disregarding the established generic name *Quercinium*. Knowlton (1899) published a description of a new species, *Quercinium lamarense*, and nothing further appeared in the literature until Platen (1908) added five new species to the genus. Since that time little has been published except for two reports by American workers on fossil woods from California, one of which was apparently identical with the modern *Quercus agrifolia*. These woods were given the generic name *Quercus*, although it would appear that less confusion would result if the genus *Quercinium* were adhered to when fossil wood is being described.

The specimen which is described in this paper was obtained from the Miocene Payette formation in the vicinity of Payette, Idaho. The only modern oaks of Idaho are confined to the southern part of the state and occur rather sparsely, but a number of fossil oak leaves have been collected from the state, and three species of *Quercites* have been described from the same locality in which this wood was found. The fossil oak herewith described belongs to the subgenus *Leucobalanus*, and the name *Quercinium album* sp. nov. is proposed by the authors, since it differs from previously described species in characters enumerated in the accompanying table. The specimen was a piece of heartwood, as seen from the presence of tyloses. The microscopic structure was fairly well preserved, and the color was orange-yellow, with white lines denoting the course of the large wood rays.

Quercinium album sp. nov.—*Transverse.*—Annual rings 1.3–1.8 mm. wide. Rays conspicuous; broad rays spaced at irregular intervals and up to 500 μ wide; small rays may continue through a ring. Transition from spring to summer wood vessels very abrupt. Vessels in spring wood very large elliptical, ovate, or sub-orbicular, measuring up to 450 μ in length and 340 μ in width, plugged with conspicuous flattened tyloses. Summer wood vessels barely visible with a hand lens, thin walled, averaging 30–75 μ in diameter.

Radial.— Pits in tracheids elliptical to orbicular, 5–7 μ up to 8–12 μ; tori sometimes oblique in reference to tracheid walls.

Tangential.— Broad rays 20–30 seriate in width; individual cells frequently hexagonal due to mutual pressure, 8–25 μ in diameter; lumen round to elliptical. Narrow rays abundant; both uniseriate and biseriate, with the uniseriate type predominating. Both types are occasionally found in the same region, but the groups are composed more often of a single type. Height of narrow rays varies from 2 to 20 cells along the grain, averaging 12 to 15, but occasionally as many as 30. The biseriate rays are usually somewhat shorter than the uniseriate.

The accompanying table contains the principal anatomical characteristics of all the species of *Quercinium* which were found in the literature. Felix (1883) mentions *Q. rossicum* Mercklin in reference to its similarity to *Q. montanum* Felix, but the authors of the present paper were unable to obtain the publication in which *Q. rossicum* was described.

The literature cited includes references to the original descriptions of all the fossil oaks referred to in this paper.

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IRREGULAR PAGINATION

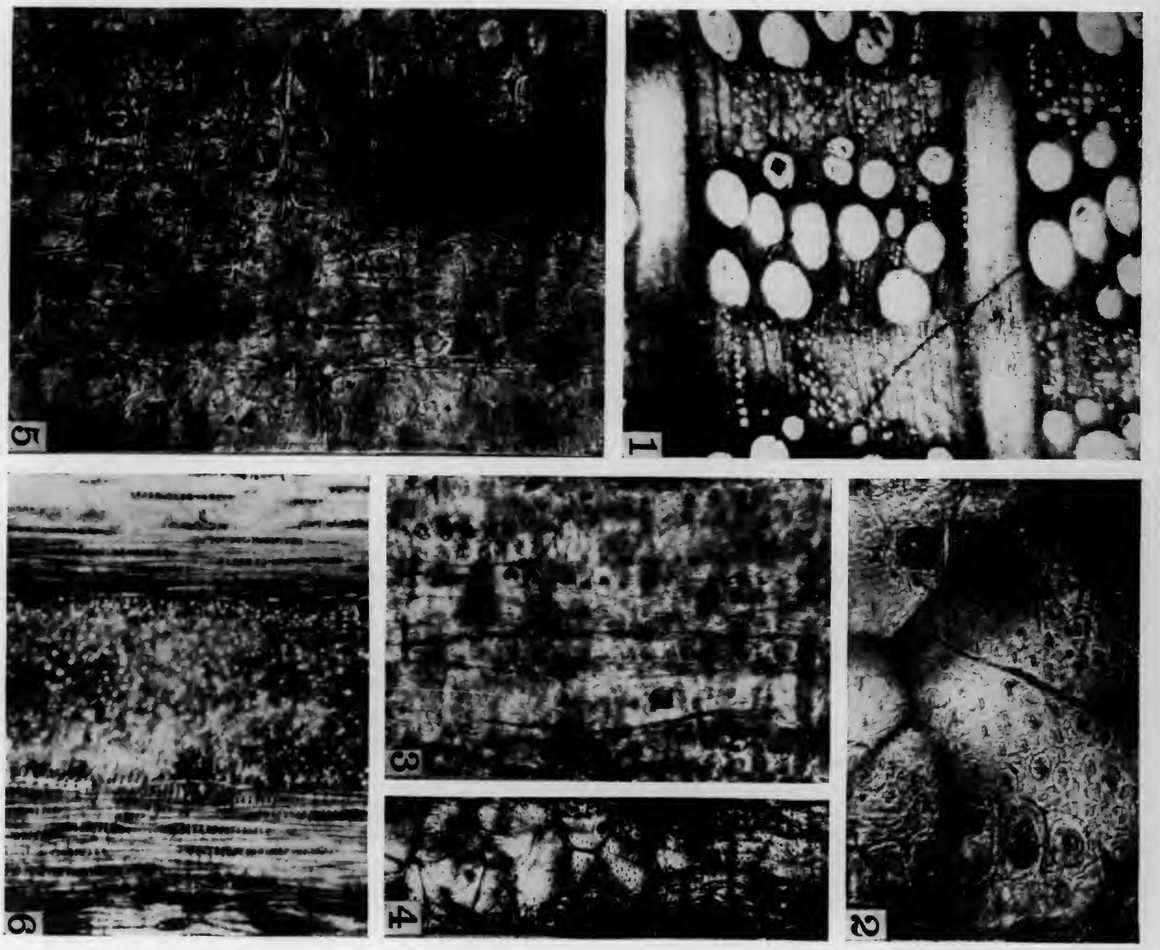

Fig. 1–6.—Fig. 1. Transverse section of *Quercinium album* showing annual rings, broad rays, and abrupt transition from spring to summer wood.—Fig. 2. Tangential section of *Q. album* showing pits in wall of a vessel.—Fig. 3. Radial section of *Q. album* showing pits in tracheid walls.—Fig. 4. Tyloses and pits in a vessel of the spring wood of *Q. album*.—Fig. 5. Wood ray of *Q. album* in radial section.—Fig. 6. Portion of broad ray and uniseriate and biseriate small rays of *Q. album*.

TABLE 1. *Anatomical characters of all described species of Quercinium found in the literature.*

Species	Spring wood	Summer wood	Rings	Small rays	Large rays	Pits	Remarks
Q. Abromeiti Platen	Vessels 212 μ to 350 μ; flattened tangentially.	Vessels 50 μ to 62 μ; not in rows.	Up to 1.5 mm. wide.		Up to 450 μ wide; 1.5 mm. apart.	7.5 μ in diameter.	A root wood.
Q. agrifolia							Identical with the modern *Quercus agrifolia.*
Q. anomalum Platen	Vessels 338 μ radially and 300 μ tangen.	Vessels 87 μ radially, 75 μ tangentially.		Up to 30 cells high. Lumen of cells 12.5 μ.	1 to 1.5 mm. apart.	Simple 17.5 μ wide. In ray parenchyma.	
Q. Böckhianum Felix	Vessels 220 μ rad. and 290 μ tan., or round and up to 320 μ in diameter.	Vessels circular, 30 μ to 50 μ. Also a few 10 μ wide among others.					Otherwise resembling *Q. compactum.*
Q. compactum Schlei.	Vessels 360 μ rad. 461 μ tan. Often with tyloses.	Vessels 80 μ by 120 μ.		Uniseriate.			Similar to the modern *Quercus lusitanicum* Lam.
Q. helictoxyloides Felix	Vessels round, up to 300 μ. Averaging 150 μ.				2.5 mm. apart, up to 400 μ wide.		A root wood.
Q. Knowltoni Felix	Vessels 500 μ radially, 210 μ tangentially, tyloses present.	Vessels 80 μ by 120 μ.		Uniseriate.	1.5 mm. apart 170 μ wide.	Small, oval, bordered.	
Q. lamarense Knowl.	Vessels round, 160 μ to 230 μ. Not in radial rows.	Vessels 100 μ to 120 μ.		Mostly pass from one duct to the next in transverse section.	10-20 cells wide. Walls thin.		Allied to *Q. Knowltoni,* but differing in size and shape of ducts.
Q. leptoticum Schlei.	Vessels large, to 570 μ radially and 390 μ tangentially.			Uniseriate, up to 25 cells high.		Large; in longitudinal rows.	
Q. Lesquereuxi Platen	Vessels 250 μ rad. and 175 μ tang. Round tyloses.	Smallest vessels 75 μ.		Up to 30 cells high. Single cells 20–25 μ to 50 μ in dia.	Up to 175 μ in width.		
Q. Marcyana Pen.	No obvious distinction between spring and summer wood.	Vessels 200 μ to 300 μ. Tangentially compressed.					Incomplete description, but resembles the modern chestnut oak.

INTENTIONAL SECOND EXPOSURE

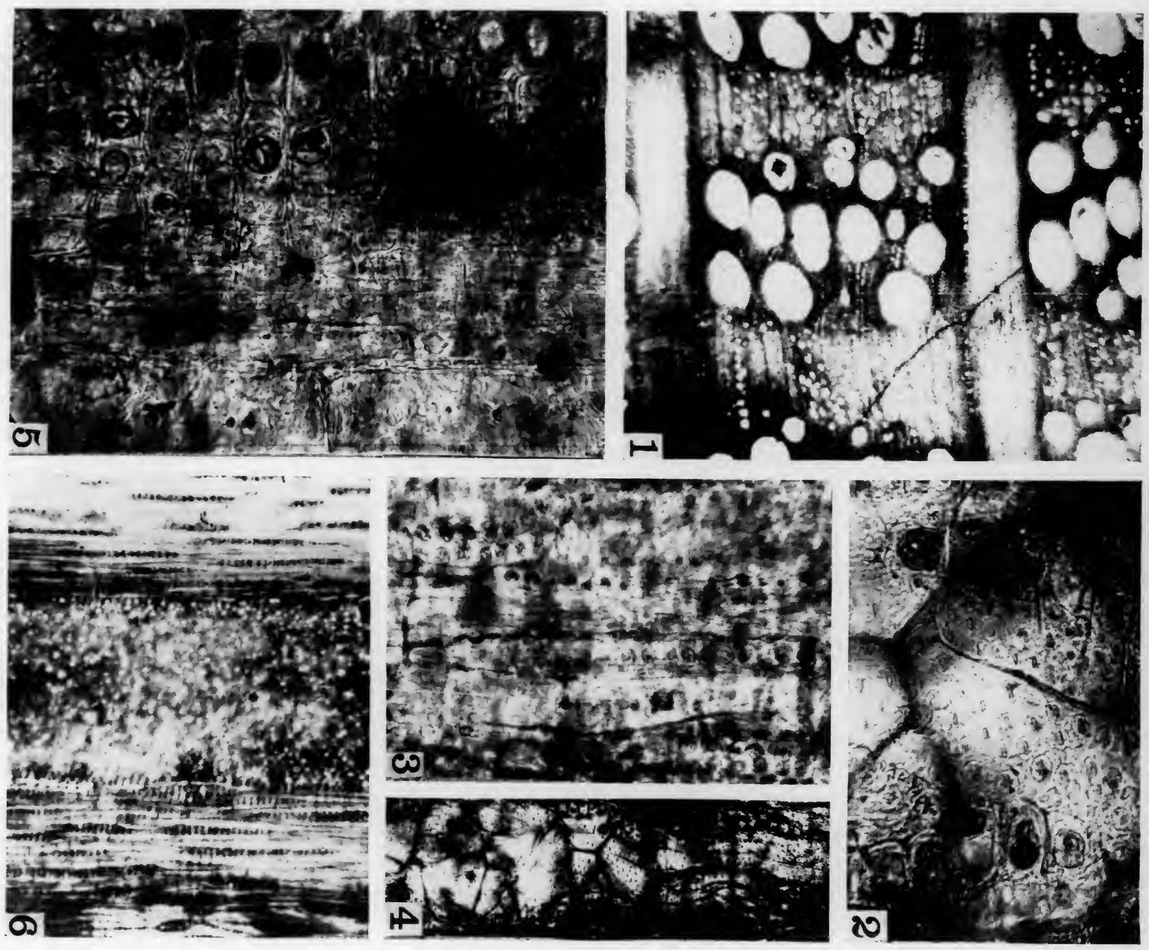

Fig. 1–6.—Fig. 1. Transverse section of *Quercinium album* showing annual rings, broad rays, and abrupt transition from spring to summer wood.—Fig. 2. Tangential section of *Q. album* showing pits in wall of a vessel.—Fig. 3. Radial section of *Q. album* showing pits in tracheid walls.—Fig. 4. Tyloses and pits in a vessel of the spring wood of *Q. album*.—Fig. 5. Wood ray of *Q. album* in radial section.—Fig. 6. Portion of broad ray and uniseriate and biseriate small rays of *Q. album*.

TABLE 1. *Anatomical characters of all described species of Quercinium found in the literature.*

Species	Spring wood	Summer wood	Rings	Small rays	Large rays	Pits	Remarks
Q. Abromeiti Platen	Vessels 212 μ to 350 μ; flattened tangentially.	Vessels 50 μ to 62 μ; not in rows.	Up to 1.5 mm. wide.		Up to 450 μ wide; 1.5 mm. apart.	7.5 μ in diameter.	A root wood.
Q. agrifolia							Identical with the modern *Quercus agrifolia*.
Q. anomalum Platen	Vessels 338 μ radially and 300 μ tangen.	Vessels 87 μ radially, 75 μ tangentially.		Up to 30 cells high. Lumen of cells 12.5 μ.	1 to 1.5 mm. apart.	Simple 17.5 μ wide. In ray parenchyma.	
Q. Böckhianum Felix	Vessels 220 μ rad. and 290 μ tan., or round and up to 320 μ in diameter.	Vessels circular, 30 μ to 50 μ. Also a few 10 μ wide among others.					Otherwise resembling *Q. compactum*.
Q. compactum Schlei.	Vessels 360 μ rad. 461 μ tan. Often with tyloses.	Vessels 80 μ by 120 μ.		Uniseriate.			Similar to the modern *Quercus lusitanicum* Lam.
Q. helictoxyloides Felix	Vessels round, up to 300 μ. Averaging 150 μ.				2.5 mm. apart, up to 400 μ wide.		A root wood.
Q. Knowltoni Felix	Vessels 500 μ radially, 210 μ tangentially, tyloses present.	Vessels 80 μ by 120 μ.		Uniseriate.	1.5 mm. apart 170 μ wide.	Small, oval, bordered.	
Q. lamarense Knowl.	Vessels round, 160 μ to 230 μ. Not in radial rows.	Vessels 100 μ to 120 μ.		Mostly pass from one duct to the next in transverse section.	10-20 cells wide. Walls thin.		Allied to *Q. Knowltoni*, but differing in size and shape of ducts.
Q. leptoticum Schlei.	Vessels large, to 570 μ radially and 390 μ tangentially.			Uniseriate, up to 25 cells high.		Large; in longitudinal rows.	
Q. Lesquereuxi Platen	Vessels 250 μ rad. and 175 μ tang. Round tyloses.	Smallest vessels 75 μ.		Up to 30 cells high. Single cells 20–25 μ to 50 μ in dia.	Up to 175 μ in width.		
Q. Marcyana Pen.	No obvious distinction between spring and summer wood.	Vessels 200 μ to 300 μ. Tangentially compressed.					Incomplete description, but resembles the modern chestnut oak.

TABLE 1. *Anatomical characters of all described species of Quercinium found in the literature.* (*Cont.*)

Species	Spring wood	Summer wood	Rings	Small rays	Large rays	Pits	Remarks
Q. montanum (Marck.) Felix	Vessels large, reaching 570 μ radially, 390 μ tangentially.			Uniseriate, up to 28 cells high.	20–30 cells in width.		Similar to *Quercus toza* Bosc. and to *Quercinium rossicum* Merck.
Q. primaevum Göpp.	Vessels 470 μ radially, 350 μ tangentially.	Gradual transition to summer wood.			1.6 mm. apart.	6.8 x 7.6 μ. Elliptical bordered pits.	Resembles the living *Quercus castanaefolia* Mey.
Q. ricardensis Web.	Vessels 112 μ by 77 μ.	Vessels slightly smaller than the spring wood.	.37 to 1.2 mm. wide.	Mostly uniseriate, 3–22 cells high. Cells 10–14 μ in diameter.	375–450 μ wide. Individual cells 7–25 wide.	3.5 to 5 μ in diameter.	
Q. Solcrederi Platen	Vessels 162 μ rad., 125 μ tang.	Vessels 63 μ radially, 50 μ tangentially.	Up to 3 mm. wide.			In vessel walls, 5 μ wide.	
Q. Staubi Felix	Vessels up to 490 μ rad., 400 μ tang., in concentric rings. Tyloses present.	90 μ rad., 80 μ tang.		5–15 cells high. 20 high occasionally.		As in *Q. primaevum*.	Calcium oxalate crystals often present.
Q. transiens Conwentz							Similar to *Q. Knowltoni* but with abrupt transition from spring to summer wood and less large rays.
Q. vasculosum Schlei.	Vessels in two rows, crowded; often with tyloses. 450 μ rad. 300–400 μ tang.	Abrupt transition to summer wood.		Uniseriate, up to 15 cells high.	3 mm. apart. Up to 30 cells in width.	As in *Q. primaevum*.	
Q. Wardi Platen	Vessels 250 μ radially; 200 μ tangentially.		Up to 8 mm. in width.			In vessel walls 10 μ wide.	An evergreen oak.
Q. album sp. nov.	Vessels up to 450 μ radially and 340 μ tangentially. Plugged with flattened tyloses.	Vessels thin walled, 30 μ to 75 μ in diameter.	1.3 to 1.8 mm. wide.	Uniseriate and biseriate, averaging 12 to 15, and up to 30 cells high.	20–30 cells wide. Cells 8–25 μ in diameter.	Elliptical to orbicular in tracheids; 5–7 μ up to 8–12 μ. Tori often oblique.	

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VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE[1]

THOMAS W. CHILDS[2]

(Accepted for publication May 1, 1936)

INTRODUCTION

Slight cultural differences within various species of wood-attacking fungi have been briefly mentioned by Long and Harsch (8) and by Fritz (3), but these workers, primarily interested in the diagnosis of decay, attached little importance to the variability they encountered. Schmitz (14) has described definite morphological and physiological differences between 4 isolations of *Fomes pinicola* (Sw.) Cke. Mounce (11), also working with *F. pinicola*, has discussed differences between cultures from a number of sources and ascribes these differences to individual variation rather than host influence. Aside from these studies, intraspecific variability in the Polyporaceae seems to have received little or no detailed attention, although the possibility of its occurrence often has been suggested.

When *Polyporus schweinitzii* Fr. was found causing a destructive root rot in 20- to 25-year-old plantings of northern white pine, *Pinus strobus* L., on unfavorable sites near Springwater, New York,[3] the possible implication of a heretofore unreported parasitic strain of either native or foreign origin could not be ignored. Cultures of this fungus, therefore, were requested from several American and foreign sources, and a number of isolations were made from diseased white pines at Springwater. A few isolations also were made from white pine and larch in New England. As this material accumulated it became increasingly apparent that there was no morphological basis for subdividing the species into local, parasitic, or host-specialized strains. Some of the cultures resembled each other rather closely, and all of them had a few characters in common, but with the exception of those taken from within a few feet of each other no two were identical, nor did those from any given host or locality manifest any special similarity.

In the past, *Polyporus schweinitzii* has been known principally as a cause of heart rot in butts of over-mature trees; with the disappearance of

[1] Presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] This study was begun during the tenure of a Morris Arboretum Fellowship in the Department of Botany of the University of Pennsylvania, and was completed at the Morris Arboretum with the assistance of a fellowship granted by the Charles Lathrop Pack Forest Education Board. The writer also wishes to acknowledge his indebtedness to Dr. H. H. York and Dr. Conway Zirkle, to Edith Adams Childs for laboratory assistance, and to the many workers who furnished cultures.

[3] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

IRREGULAR PAGINATION

virgin stands it was expected to become relatively unimportant. Its destructiveness at Springwater and in a Douglas fir plantation near Biltmore, North Carolina, (6) suggests, however, that it may constitute a serious obstacle to the use of coniferous species in artificial reforestation, at least on certain sites. An investigation of its variability has, therefore, been undertaken to determine to what extent specialized strains may be responsible for damage to young trees. Certain preliminary observations are presented here. Final conclusions will be possible only when the results of field studies, already inaugurated, become available.

SOURCES AND DESCRIPTIONS OF CULTURES

It was the writer's practice to make several isolations from each sporophore or infected tree from which a culture of the fungus was desired. More than 100 isolations, representing 25 sporophores and 9 trees, were thus secured and cultured on nutrient agar under closely comparable conditions. With 2 exceptions, isolations from any given tree or sporophore were indistinguishable from one another, but differed more or less distinctly from those from any other source. It, therefore, was assumed that similar isolations from the same source were derived from the same individual mycelium, *i.e.*, were members of the same clone, and that dissimilar isolations were derived from genetically different mycelia. These mycelia, together with those obtained from other workers, will be referred to by the following numbers:

1 to 30, inclusive. From *Pinus strobus*; Springwater, N. Y.
31. From *Pinus strobus* (lesion on 0.5 cm. root of an 8-year-old planted tree); Honeoye Lake, N. Y.
32. " " " ; Weston, Ontario.
33. " " " ; Lake Timagami, Ontario.
34. " " " ; Harvard Forest, Petersham, Mass.
35. " " " ; Brattleboro, Vt.
36. " " *silvestris*; Stralsund, Germany.
37. " " " ; Skjaerningsfjell, Ringebii, Norway.
38. " " " ; Great Britain.
39. " " *rigida*; Medford, N. J.
40. " " *mughus;* Central Experimental Farm, Ottawa, Ontario.
41. " *Picea canadensis*; Lake Timagami, Ontario.
42. " " *sitchenis*; Moresby Island, British Columbia.
43. " " " ; Gahrenberg, Germany.
44 and 45. From *Pseudotsuga taxifolia*; Berlin, Germany.
46. From *Larix europea*; Island of Visingsö, Lake Vetter, Sweden.
47. " " *laricina*; Franconia, N. H.
48. " *Thuja plicata*; Vancouver, British Columbia.

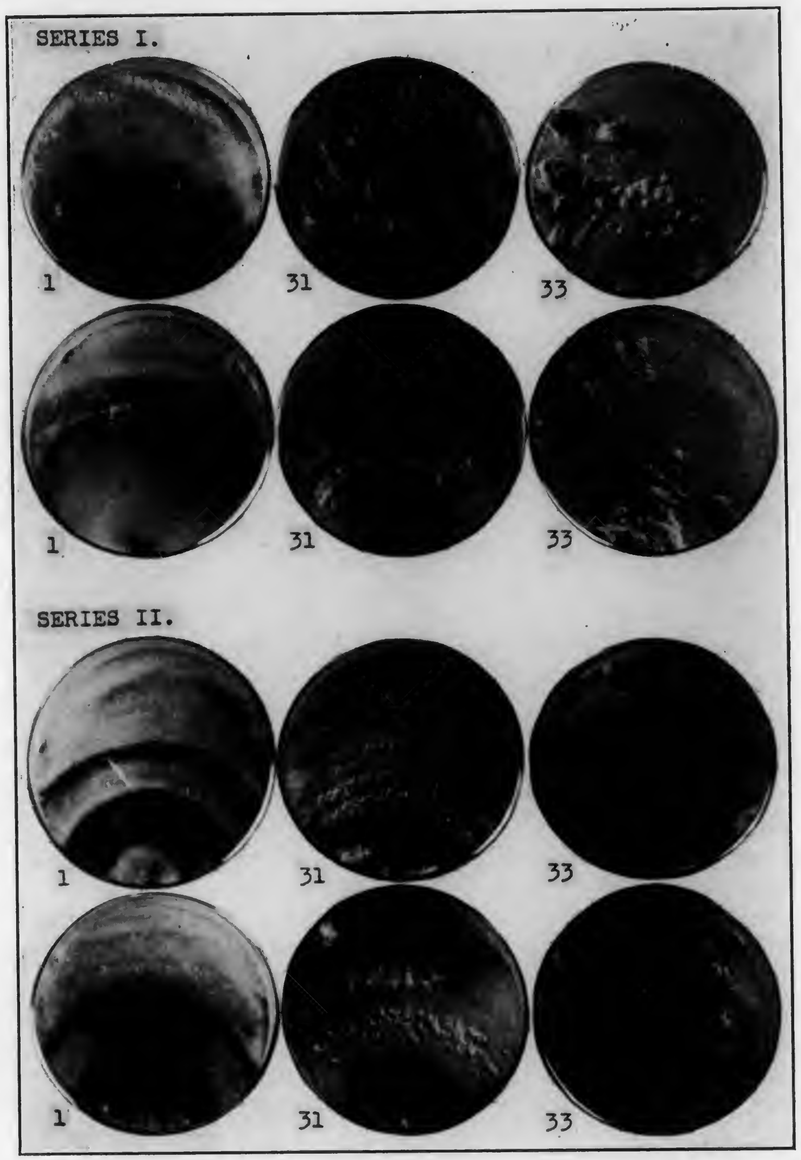

FIG. 1. Cultures showing differences between individual mycelia and between cultures of the same mycelium exposed to different environments, and similarities between parallel cultures of each mycelium. Numerals refer to the numbers given the various mycelia on pages 30 and 32.

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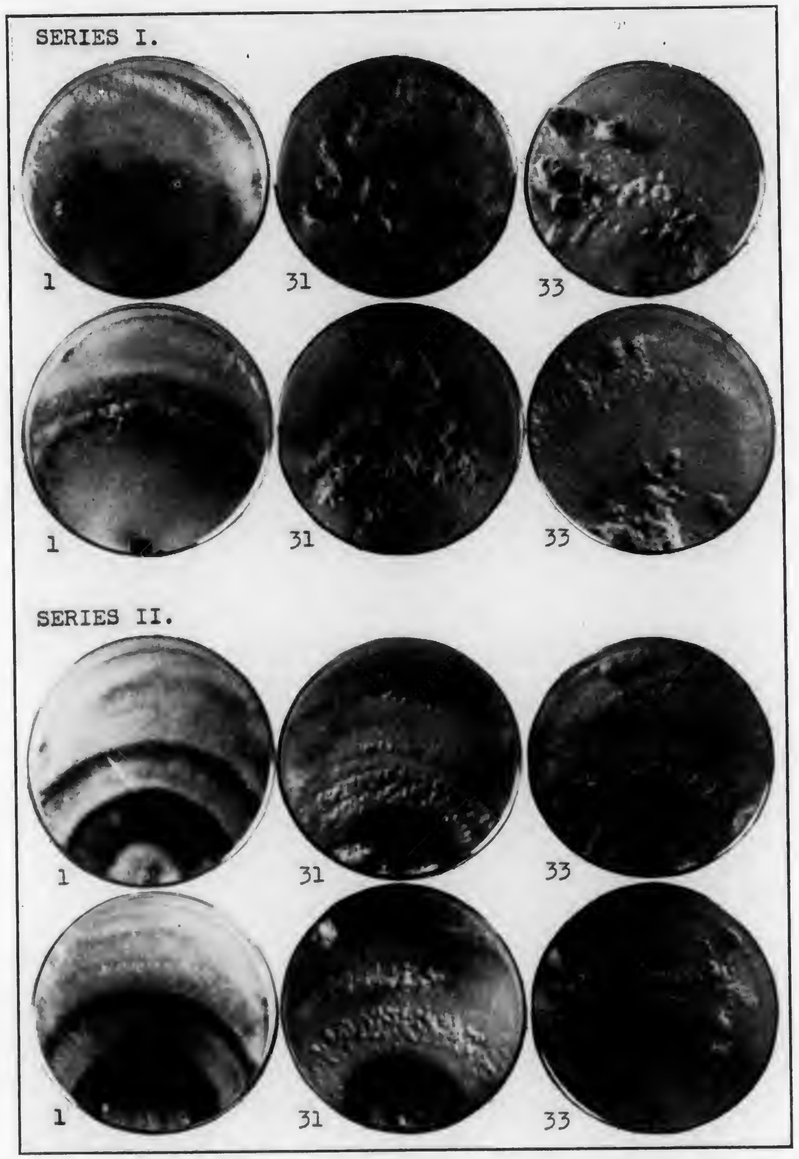

FIG. 1. Cultures showing differences between individual mycelia and between cultures of the same mycelium exposed to different environments, and similarities between parallel cultures of each mycelium. Numerals refer to the numbers given the various mycelia on pages 30 and 32.

INTENTIONAL SECOND EXPOSURE

49. Host not specified; Timagami Forest Reserve, Ontario.
50. Host not specified; Kyota, Japan.

One isolation of each mycelium was maintained as a stock culture from which all subsequent cultures of that mycelium, except when otherwise noted, were derived.

Cultures of these mycelia were under the writer's observation for periods of from 6 to 20 months, and in no instance was any alteration of cultural characters detected. At the same stage of development, cultures of any given mycelium differed in appearance only when they had been subjected to different environments. This is shown in figure 1, where growth characters of each of 3 representative mycelia are seen to be consistent in duplicate cultures raised under the same conditions (*i.e.,* in the same series) but are not consistent in cultures of the same mycelium in different series. The appearance of a given mycelium cultured in a given environment was determined entirely by the inherent nature of that particular individual mycelium and by the factors of that particular environment; *i.e.,* the cultural history of the material from which transfers were made had no appreciable effect upon the characters of the derived cultures.

Schmitz (14) and Mounce (11) have described and illustrated the line of demarcation that usually develops when different mycelia of *Fomes pinicola* approach each other on the same artificial substratum. An apparently identical reaction occurs between mycelia of *Polyporus schweinitzii.* When isolations from different sources were subcultured on the same slant or plate of nutrient agar, a well-defined dark line about 1 mm. wide usually appeared between them soon after the advancing margins had come in contact. This line did not form between subcultures taken from a single isolation or from similar isolations from any single source, nor did it form when monosporous mycelia were paired with other monosporous mycelia of either the same or different ancestry.

Where not otherwise specified, cultures were made on nutrient agar consisting of 20 g. of malt extract (either Fleischmann's Diamalt or Trommer's Diastasic) and 20 g. of agar per litre of distilled water. Sterilization was effected by autoclaving for 15 minutes at a pressure of 15 pounds. Growth was somewhat more luxuriant and fruiting was more frequent on media made with Diamalt. Trommer's extract was variable, some bottles being caramelized to a much greater extent than others, but the line of demarcation formed between mycelia from different sources was generally darker and more distinct when this brand of nutrient was used. In other respects these two brands of malt extract did not differ perceptibly in their effect on growth characters.

As Fritz (3) has remarked, *Polyporus schweinitzii* is quite sensitive to

FIG. 2. Cultures showing differences between individual mycelia. Note sporophores in Nos. 16 and 41.

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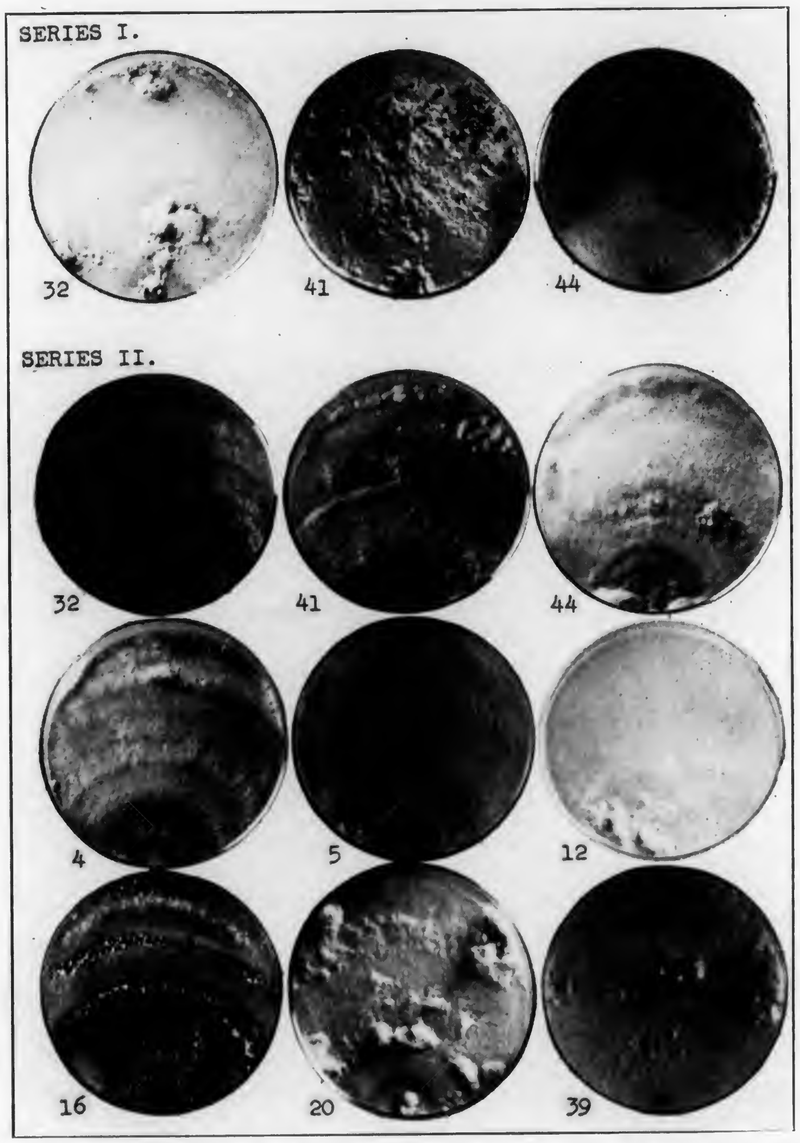

FIG. 2. Cultures showing differences between individual mycelia. Note sporophores in Nos. 16 and 41.

INTENTIONAL SECOND EXPOSURE

TABLE 1.—*Descriptions of some cultures of representative mycelia*

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
1.	I.	Sienna to olive brown; short pile (occasionally with 1 or 2 indistinct concentric zones of longer and darker pile).		Pale and sparse.	Indistinct. (Fig. 1).
	II.	Dark greenish-brown; uniform and moderately long pile.	Brown; uniform and moderately long pile.	Light red brown to lemon yellow; uniform and moderately long pile.	Quite distinct. (Fig. 1).
2.	I.	Yellow and light brown; fairly even mat.		Pale and sparse.	None.
	II.	Mostly greenish or reddish brown, with some lemon yellow to creamy yellow; regular and moderately long pile (occasionally slightly massed, tufted, or fluffy).			Fairly distinct.
3.	I.	Red brown; numerous very small tufts (some areas with short and fairly regular pile).		Lemon yellow.	Indistinct.
	II.	Light orange brown; fairly regular pile becoming very slightly and uniformly tufted.	Pale to lemon yellow; sparse to moderately long and irregular pile becoming slightly fluffy near edge of culture.		Fairly distinct.
4.	I.	Yellow brown (sometimes with one or two concentric zones of lighter yellow); short, matted pile.		Pale to lemon yellow.	Fairly distinct.
	II.	Olive to greenish or reddish brown; moderately long and uniform pile.			Fairly distinct. (Fig. 2).
5.	I.	Pale lemon yellow to reddish brown; an uneven mat.			Vague.
	II.	Yellow to orange; moderately long and irregular pile (some concentric zones of tufted, compacted, or fluffy growth.			Generally indistinct. (Fig. 2).
7.	II.	Mostly rather pale but with 2 or 3 fairly distinct concentric zones of lemon yellow to light brown; sparse except in regions of darker color, where regular pile is fairly well developed.			Fairly distinct.
12.	II.	Lemon yellow; short and irregular pile with some semicompact or slightly fluffy growth.	Pale and sparse.	Very pale; very sparse.	None. (Fig. 2).
18.	II.	Zones of reddish brown, short, and regular pile alternate concentrically with zones of dark yellow to orange, longer (sometimes slightly fluffy) pile.		Lemon yellow; moderately long and fairly regular pile (sometimes slightly fluffed).	Quite distinct.

TABLE 1.—(*Continued*)

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
31.	I.	Dark, mottled, yellow brown; irregular, matted pile with frequent fluffy growth.		Pale and sparse.	None. (Fig. 1).
	II.	Greenish to reddish brown; rather long and irregular pile.	Dark creamy yellow; small to medium large rounded tufts.	Pale to yellow green (with some reddish brown); sparse, irregular, or fluffy.	Fairly distinct. (Fig. 1).
42.	I.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with numerous long, cobwebby, lemon yellow to brown wefts or fluffy masses.	None.
	II.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with zones of loose, creamy wefts and some creamy to yellow green fluffs at edge of culture.	Rather vague.
44.	I.	Pale to yellow to reddish brown; very thin mat with short, sparse, pile (some lemon yellow, fluffy growth at edge of culture).			None. (Fig. 2).
	II.	Dark cream to orange; irregular (sometimes fluffy) pile.	Light to dark yellow green; pile irregular (fluffy in some central areas and near edge of culture).		Indistinct. (Fig. 2).
47.	I.	Yellow brown to reddish brown; short, irregular, pile and occasional small yellow fluffs.	Generally pale and sparse but usually with some pale lemon yellow growth near the edge of the culture.		Vague.
49.	II.	Greenish yellow, orange, and reddish brown; fairly long and regular pile (with a few small tufts).	A narrow zone of creamy, moderately fluffy growth.	Creamy to yellow green; sparse pile with some fairly well developed fluffy growth.	Rather indistinct.

light, growing more slowly and displaying darker and more varied coloration when exposed, even for very short periods, to indirect daylight than when kept in darkness. High humidity stimulates the development of fluffy superficial growth. Temperature, concentration and kind of nutrient, etc., also affect the appearance of cultures. The continuous nature of these environmental variables and the apparently great number of hereditary factors involved make it impracticable to describe in detail all the mycelia studied. Partial descriptions of a few representative mycelia cultured in Petri dishes are presented in table 1, however, to indicate the range and kind of cultural variations encountered during the study. In this table, and in figures 1 and 2, series I consists of cultures stored at a constant temperature of 22°[4] and exposed to electric light, but never to daylight, for a few seconds every 12 hours, while series II consists of cultures stored at room temperature (10° to 25°) and exposed to diffuse daylight for a few seconds every 4 or 5 days. Descriptions of series I were taken on the 19th day and photographs on the 40th day of growth. Descriptions and photographs of series II were taken on the 29th day of growth. From 2 to 20 cultures were made of each mycelium in each series. Cultural characters of each mycelium in each series were consistent in all cases where not otherwise indicated.

From the descriptions in table 1, and from the photographs in figures 1 and 2, it is apparent that differences between mycelia may sometimes be large enough to make difficult the diagnosis of wood decay by cultural methods. Identification may be facilitated by culturing on agar slants in ordinary test tubes and by storing cultures where they will be exposed to daylight of low intensity, since these conditions promote the formation of the more or less leathery mat and hasten the appearance of the "range of yellow tints" (3) typical of this species, but, even under these conditions, occasional mycelia are not readily identifiable on the basis of their cultural characters. The writer has not investigated the microscopic features of the various mycelia to a sufficient extent to determine their reliability as criteria of specific identity. Hasty and superficial observations indicate, however, that differences in microscopic characters are neither so great nor so frequent as are differences in gross cultural characters.

A number of mycelia were noticeably destructive to the agar, in some spots lowering the level of the surface 3 mm. or more below that of the rest of the culture (Fig. 3, 40K). Such lowering usually was confined to the region near the inoculum, but sometimes occurred in other portions of the culture. This destructive effect upon the agar, and the particular region in which destruction was visible, were quite as characteristic of certain mycelia as were the color and character of growth in culture. In cultures

[4] All temperatures recorded in this paper are in °C.

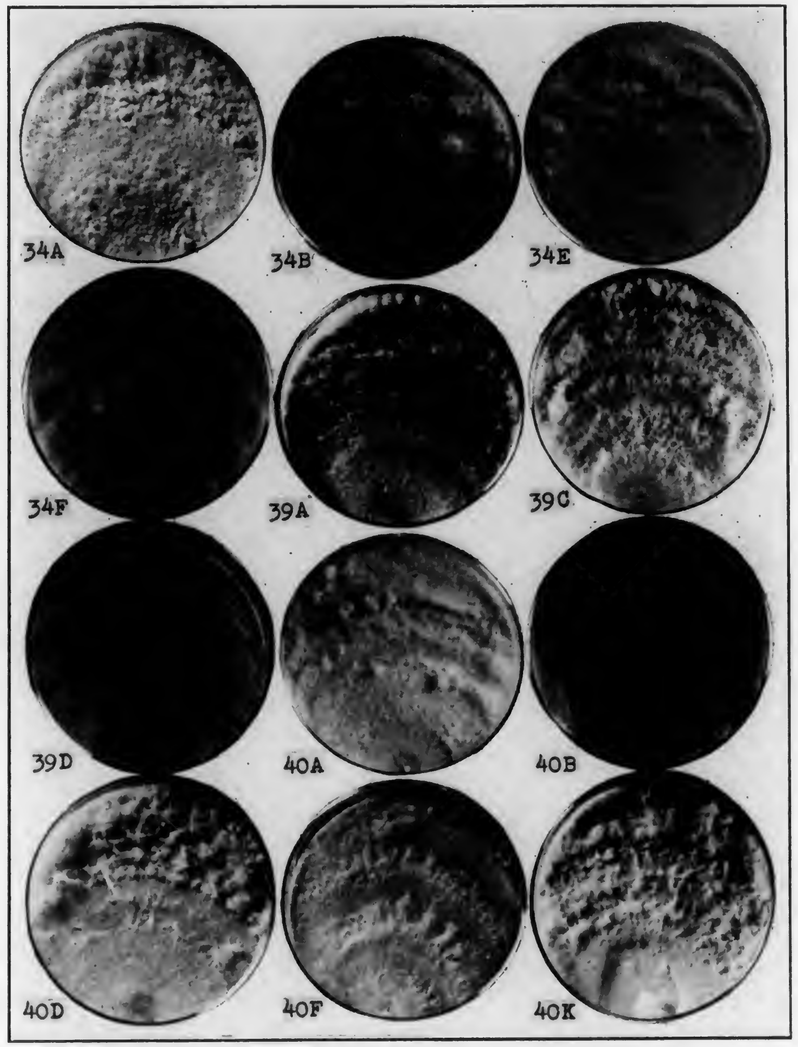

FIG. 3. Cultures showing individual differences between monosporous mycelia. Note sporophores in No. 40B and destruction of agar near inoculum in No. 40K.

light, growing more slowly and displaying darker and more varied coloration when exposed, even for very short periods, to indirect daylight than when kept in darkness. High humidity stimulates the development of fluffy superficial growth. Temperature, concentration and kind of nutrient, etc., also affect the appearance of cultures. The continuous nature of these environmental variables and the apparently great number of hereditary factors involved make it impracticable to describe in detail all the mycelia studied. Partial descriptions of a few representative mycelia cultured in Petri dishes are presented in table 1, however, to indicate the range and kind of cultural variations encountered during the study. In this table, and in figures 1 and 2, series I consists of cultures stored at a constant temperature of 22°[4] and exposed to electric light, but never to daylight, for a few seconds every 12 hours, while series II consists of cultures stored at room temperature (10° to 25°) and exposed to diffuse daylight for a few seconds every 4 or 5 days. Descriptions of series I were taken on the 19th day and photographs on the 40th day of growth. Descriptions and photographs of series II were taken on the 29th day of growth. From 2 to 20 cultures were made of each mycelium in each series. Cultural characters of each mycelium in each series were consistent in all cases where not otherwise indicated.

From the descriptions in table 1, and from the photographs in figures 1 and 2, it is apparent that differences between mycelia may sometimes be large enough to make difficult the diagnosis of wood decay by cultural methods. Identification may be facilitated by culturing on agar slants in ordinary test tubes and by storing cultures where they will be exposed to daylight of low intensity, since these conditions promote the formation of the more or less leathery mat and hasten the appearance of the "range of yellow tints" (3) typical of this species, but, even under these conditions, occasional mycelia are not readily identifiable on the basis of their cultural characters. The writer has not investigated the microscopic features of the various mycelia to a sufficient extent to determine their reliability as criteria of specific identity. Hasty and superficial observations indicate, however, that differences in microscopic characters are neither so great nor so frequent as are differences in gross cultural characters.

A number of mycelia were noticeably destructive to the agar, in some spots lowering the level of the surface 3 mm. or more below that of the rest of the culture (Fig. 3, 40K). Such lowering usually was confined to the region near the inoculum, but sometimes occurred in other portions of the culture. This destructive effect upon the agar, and the particular region in which destruction was visible, were quite as characteristic of certain mycelia as were the color and character of growth in culture. In cultures

[4] All temperatures recorded in this paper are in °C.

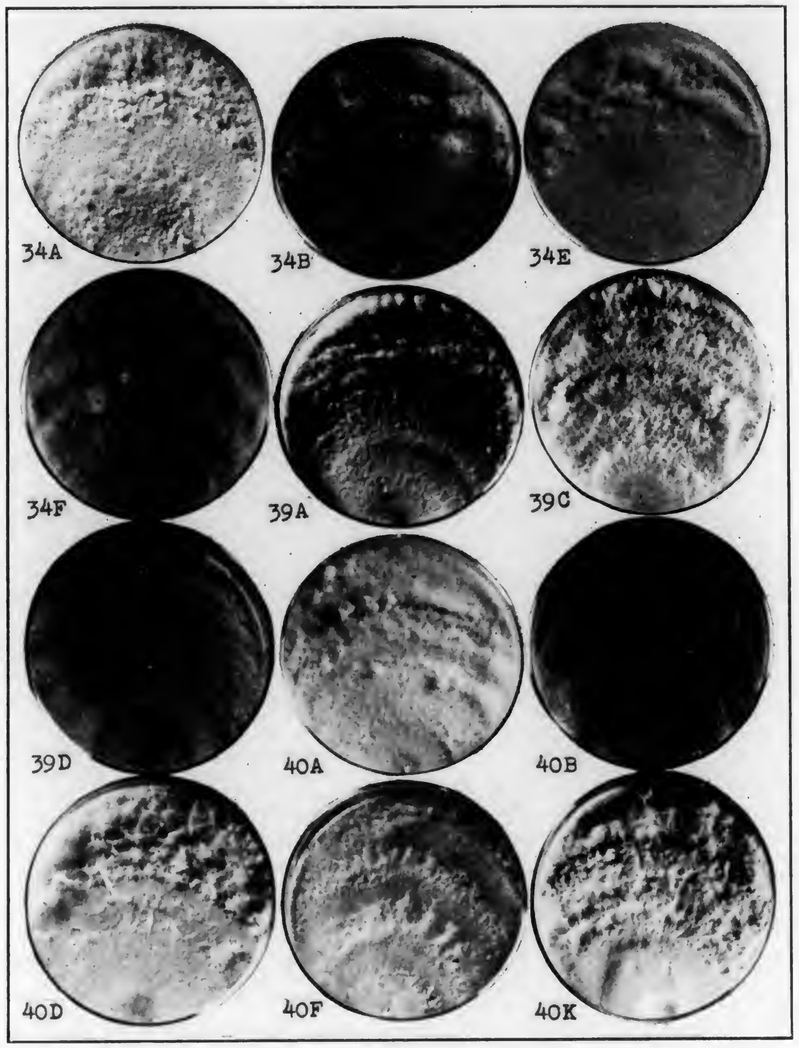

FIG. 3. Cultures showing individual differences between monosporous mycelia. Note sporophores in No. 40B and destruction of agar near inoculum in No. 40K.

INTENTIONAL SECOND EXPOSURE

of mycelia that did not alter the level of the surface of the substratum the agar retained its original gelatinous consistency until dry, but mycelia that caused any perceptible lowering of the agar surface also rendered the rest of the agar definitely crumbly, even in regions not adjacent to spots of visible destruction. Schmitz (14) has shown that some mycelia of *Fomes pinicola* secrete larger quantities of certain enzymes than are produced by other mycelia of the same species. The phenomenon just described suggests that mycelia of *Polyporus schweinitzii* also differ in enzyme production.

In only one instance during the entire study did repeated isolations from the same source fail to yield identical cultures. Two isolations each of Nos. 23 and 24 were made from a single sporophore. These mycelia differed so markedly in appearance as to be distinguishable from each other during very early stages of development. No. 23 was in no way noteworthy, displaying the usual cultural characters of the species and differing from most of the other mycelia only in relatively minor details. No. 24, however, became established very rapidly at ordinary temperatures and quickly filled the space above the agar slants with a permanently fluffy mass of hyphae. Portions of this mass ranged from a faint lemon yellow to a very light red brown, but most of it remained white throughout the life of the culture. In many respects this individual resembled some of the single-spore cultures described in a subsequent section.

No. 47, the only mycelium found in more than one individual host, was isolated several times from each of 3 wind-thrown larches separated from each other by distances of 20 to 30 feet. Since previous experience had shown that even neighboring hosts of the same species usually were infected by different mycelia, isolations from each of these trees were kept under close observation for about 6 months. At no time during this period were any greater differences detected between them than were to be found between parallel cultures derived from any single mycelium, nor was a line of demarcation formed when they grew together on a common substratum. Fruiting bodies have not been produced by these isolations and the possibility that they represent different mycelia belonging to a definite and perhaps specialized strain, therefore, has not been tested. Their homogeneity suggests, however, that a single mycelium has spread vegetatively to trees near the one originally infected.

RATE OF GROWTH ON NUTRIENT AGAR

During the growth of the cultures in series I, described in the preceding section, the radius of each culture was determined every 12 hours by measuring along the diameter of the Petri dish from the edge of the inoculum to the margin of the colony. Table 2 contains growth data derived from these measurements for 15 representative mycelia. (Nos. 4a and 35a were started

TABLE 2.—*Growth of representative mycelia in series I*

Mycelium No.	Days since establishment of cultures								Diff. between max. and min. on 8th day
	1	2	3	4	5	6	7	8	
	Average radius of mycelium—mm.								*mm.*
1.	11	17	23	28	35	43	51	58	7
2.	10	16	21	27	33	39	47	55	2
3.	10	16	22	29	37	45	53	61	8
4.	10	16	21	28	34	41	48	53	13
4a.	11	17	23	29	34	40	46	54	9
5.	10	16	23	30	37	44	52	60	3
31.	10	16	22	28	36	44	52	60	3
35.	10	15	21	27	33	40	47	55	3
35a.	10	15	21	27	34	40	48	56	3
38.	9	18	28	37	46	55	64	74	3
40.	8	11	14	18	22	25	30	34	13
41.	11	18	25	33	42	51	60	69	4
42.	12	21	30	38	46	55	64	74	6
43.	12	19	27	33	40	50	59	68	2
46.	13	22	30	38	46	54	62	70	2
48.	10	15	21	28	34	42	49	56	4
50.	10	16	21	27	34	41	49	56	6

one week later than the rest of the series, but were otherwise identical in origin and treatment with Nos. 4 and 35, respectively). Averages for each mycelium are based on 4 cultures of that mycelium. The principal difficulty encountered in studies of growth rates in this species was the difference in time required by the various mycelia for establishment on new substrata. In most instances, this difference was correlated with the character of the growth in cultures from which transfers were made. Other factors being equal, establishment was rapid when the transferred material included fluffs of loose aerial hyphae; less so when the aerial growth was compacted or matted; and slowest when the projecting hyphae were sparse and short. Mycelia that were to be transferred for comparative purposes were invariably cultured under the same conditions, but, because of inherent morphological differences, it was impossible to secure the same type of material for transfer from different mycelia. For this reason, the age of each mycelium in table 2 is computed from the time when the 4 cultures of that mycelium attained an average radius of 5 mm. (arbitrarily taken to indicate establishment) instead of from the time the transfers were made. Averages have been calculated, where necessary, by straight-line interpolation between the original semi-daily averages. The close correspondence between Nos. 4 and 4a, and between Nos. 35 and 35a, shows that, within rather wide limits, the

age of the agar is not an important factor, and that the arrangement of the data in this table may therefore be considered legitimate.

Differences between mycelia are clearly apparent in table 2. It is evident that Nos. 38, 41, 42, 43, and 46 have attained a greater radius and are growing more rapidly than the other mycelia, and that the growth rate of No. 40 is relatively low. Relative growth rates of mycelia cultured at higher or lower temperatures than series I were, however, frequently not consistent with the classification suggested by this table. For example, at 27° No. 43 grew more rapidly than No. 42, while at room temperature (Series II) No. 42 grew more rapidly than did No. 43. The cultures of No. 48 included in series II increased their average radius from 8 to 84 mm. in 14 days, while the cultures of No. 50 in the same series increased from 7 to only 65 mm. during the same period: at 27° the growth of No. 50 was appreciably more rapid than was that of No. 48. In every case where 2 mycelia differed significantly in growth rate at the extremes of the temperature range (app. 10° to 27°) to which they were exposed, but not at one or more of the intermediate temperatures, the mycelium that grew relatively rapidly at one extreme grew relatively slowly at the other. These observations indicate a difference between mycelia either in the form of the temperature-growth curve, or in the location of the maximum ordinate of this curve (*i.e.*, the optimum), or both.

Inclusion of the 3 mycelia from spruce in the group of rapidly growing individuals in table 2 is probably the result of coincidence rather than of any host-correlated relationship, since these 3 mycelia differed rather widely from one another in growth rate at other temperatures. Mycelia from Springwater, with one or two exceptions, maintained their relative rates of growth more consistently at the different temperatures to which they were subjected, and resembled each other more closely in absolute growth rates than did mycelia from other sources. This tendency toward similarity, even if not fortuitous, does not necessarily imply the existence of a specialized strain at Springwater. It may indicate only the closer degree of relationship that might logically be expected between isolations from a limited area as compared with isolations from widely separated points.

EFFECT OF ACIDITY ON GROWTH

Nutrient solutions adjusted to 9 different acidities were prepared by mixing 3 parts of 3 per cent Trommer's malt solution with 2 parts of KH phthalate buffers compounded according to the formulas of Clark and Lubs (2). The buffered solutions were placed in 38 × 200-mm. test tubes (app. 65 cc. of solution per tube) and autoclaved 15 min. at 15 lbs. pressure. The acidity of a sample from each lot was then determined potentiometrically, and the remaining tubes were inoculated with various representative mycelia. One culture at each pH was made of each mycelium, except No. 1, which was cultured in duplicate. Twenty-three days after inoculation the depth of the mycelium and the acidity of the medium in each tube were determined (Table 3). Since in no case did the change in pH during the growth of the fungus exceed 0.2, and since the 2 cultures of No. 1 at each acidity were reasonably consistent, the data in this table are considered approximately indicative of the effect of acidity on the growth of these mycelia. It will be seen that in every instance the optimum pH for growth was greater than 4.0 and less than 5.4, and in most cases probably lay between 4.2 and 5.0. Within these limits, however, the acidity permitting maximum growth was somewhat different for different mycelia. For example, Nos. 31 and 40 obviously made their best growth in more acid media than did Nos. 49 and 50.

TABLE 3.—*Effect of acidity on growth of mycelium of Polyporus schweinitzii*

Initial pH of buffered nutrient	Mycelium No.									Final pH
	1	1	8	31	40	42	47	49	50	
	Depth of mycelium—mm.									
2.5	9	10	5	5	8	8	6	6	15	2.6
3.0	18	18	13	12	15	15	10	10	28	3.0 to 3.2
3.5	26	27	28	30	22	34	16	25	45	3.6 to 3.7
4.0	40	43	41	44	45	46	37	39	52	4.2
4.3	42	44	50	45	46	52	50	42	52	4.5
4.7	44	46	55	39	40	50	43	42	55	4.8 to 4.9
5.1	43	43	53	36	7	48	39	46	55	5.2
5.4	22	21	28	17	9	20	20	34	33	5.4 to 5.6
5.7	10	10	10	7	4	11	7	12	15	5.6 to 5.9

RATE OF DECAY OF WOOD BLOCKS IN VITRO

Thirty-two white pine blocks (all from the same tree) approximately $\frac{7}{8}'' \times \frac{7}{8}'' \times 4''$ were air-dried in the laboratory for several weeks, given individual numbers, weighed to the nearest half-gram, placed in 38 × 200-mm. test tubes half full of water, and autoclaved 30 min. at a pressure of 15 lbs. They were then transferred to similar tubes, each of which contained a pure culture of the fungus growing actively in about 25 cc. of 2 per cent malt-extract solution, and stored in darkness at 22°. Two months later an additional 30 cc. of sterile distilled water was added to each tube. After a total incubation period of nearly 9 months the blocks were removed from the tubes, air-dried for 3 weeks, and reweighed. Average losses in weight, expressed as percentages of the original air-dry weights, were as follows:

Heartwood	
9 blocks inoculated with No. 1	$4.9 \pm 0.7\%$
7 " " " " 39	$3.7 \pm 0.7\%$
Difference	$1.2 \pm 1.0\%$
Sapwood	
9 blocks inoculated with No. 1	$17.7 \pm 1.0\%$
7 " " " " 39	$24.9 \pm 2.7\%$
Difference	$7.2 \pm 2.4\%$

The difference between the 2 sets of heartwood samples cannot be considered significant, since it is only slightly greater than its probable error.[5] The difference between the 2 sets of sapwood samples, however, is 3 times its probable error, and in spite of the small basis it may, therefore, be concluded that there probably is a real difference in the ability of these 2 mycelia to attack sapwood under the conditions of this experiment. Schmitz (14) presents evidence of similar differences between mycelia of *Fomes pinicola*. In his studies, as in the present instance, the relative order of destructiveness in heartwood is not the same as that in sapwood.

SPOROPHORES IN ARTIFICIAL CULTURE

Eleven mycelia, including isolations from both sporophore tissue and decayed wood, fruited *in vitro* during the course of these studies. Nine of the 11 fruited under conditions to which most or all of the 39 sterile mycelia also were exposed, while the remaining 2 produced sporophores only under conditions to which less than half of the other mycelia were subjected. A few of the fertile mycelia fruited commonly: in others, fruiting was rare or sporadic.

In the series of wood-block cultures described in the preceding section, No. 39 formed sporophores consistently on sapwood but not on heartwood. It also fruited several times on white-pine sawdust and at the surface of cultures growing in a 2 per cent solution of malt extract. The only other mycelium cultured on these media was No. 1, which failed to fruit on any of them.

On Trommer's malt agar, Nos. 39 and 50 produced one sporophore each. Nos. 23, 30, 34, and 41 occasionally formed a few sporophores, and No. 40 fruited rather commonly. On this medium, sporophores usually appeared only in rather old cultures. Fruiting, therefore, might have been less sporadic had it been possible to retain the cultures longer.

Diamalt agar was apparently a somewhat better medium for sporophore

[5] Standard deviation $= \sqrt{\frac{\Sigma(fd^2)}{n-1}}$

Probable error of average $= \frac{0.6745 \text{ S.D.}}{\sqrt{n}}$

Probable error of difference $= \sqrt{P.E._a^2 + P.E._b^2}$

production. No. 1 fruited rarely; Nos. 9, 13, 23, and 41 fruited occasionally; and No. 16 fruited fairly regularly on this medium. No. 34 never produced sporophores in test-tube cultures but did so invariably in Petri dishes except when the layer of agar was quite thin. The most consistent and prolific fruiting encountered during the entire study was afforded by No. 40. This mycelium commonly produced sporophores in test-tube cultures: in Petri dishes, sporophore rudiments appeared soon after the surface of the agar was overgrown and functioning sporophores were often present in cultures only 3 weeks old.

The largest sporophores were those of Nos. 39 and 50, which averaged 1 cm. in diameter and ranged from 5 to 15 mm. in thickness: the smallest were those of No. 40, which averaged 3 mm. in diameter and 2 mm. in thickness. Color varied from yellow (Nos. 34 and 40) to reddish brown (No. 50) or dark brown (No. 39). Neither stipes nor recognizable pilei were present in any instance. Sporophores that developed above the culture medium consisted principally of short spines (produced by the majority of the fertile mycelia), small and disarticulated lamellae (Nos. 34 and 40), or longer hydnoid processes, frequently once- or twice-dichotomized (formed only by No. 39). Pores occurred infrequently, and then only in sporophores borne laterally, as on wood blocks and vertical agar slants, or vertically, as in inverted Petri dishes. Average pore dimater varied from less than 0.5 mm. in No. 40 to slightly more than 1 mm. in No. 39. Small pores were approximately circular but the larger ones were quite angular. Young fruiting bodies that had recently become spiny or lamellate could sometimes be induced to form pores by reversing their orientation with respect to gravity. Neither sporophore production nor pore formation appeared to depend on exposure to daylight. The microscopic structure of hymenia in artificial culture did not differ essentially from that of hymenia developed under natural conditions. Sporophores formed by different mycelia displayed some microscopic dissimilarities—for example, cystidia of No. 34 were relatively large and sometimes terminally swollen, while those of No. 40 were smaller and of more nearly uniform diameter throughout. These variations, however, were no greater than those occurring normally in "wild" sporophores. It seems probable that the well-known variability of the sporophores of many species of Polyporaceae under natural conditions (9, 15) is simply another example of differences between individuals.

MONOSPOROUS AND POLYSPOROUS MYCELIA

Basidiospores of *Polyporus schweinitzii* are so small and fragile that it is extremely difficult to secure uninjured single spores by Hanna's dry needle method (4), even with micromanipulation apparatus. Progressive dilution of material from spore prints is much less laborious, but the serviceability

of this method is impaired by the persistence with which many of the spores remain aggregated in small groups throughout the process of dilution. The procedure devised by Mounce (11),[6] however, proved quite satisfactory and in a slightly modified form was followed in obtaining all single-spore cultures except those from No. 39, which were secured by dilution. Polysporous cultures were obtained by scraping an uncontaminated spore print with a sterile spear-pointed needle and planting the material thus obtained on a slant of nutrient agar. Both monosporous and polysporous cultures were derived in all cases from spores produced by pure cultures of the fungus.

Fresh spores germinated readily, and numerous monosporous cultures were obtained from Nos. 34, 39, 40, and 50. These monosporous cultures resembled the stock mycelia in many respects. The mat developed on the surface of the agar seemed, however, generally thinner and more delicate, and superficial growth was often much fluffier. The following, cultured under the same conditions as series II, are fairly representative (Figure 3):

No. 34A.[7] Orange brown and rather irregular pile with numerous short, fluffy, pale lemon yellow tufts (Fig. 3).

No. 34B. Growth sparse and pale near inoculum; becoming pale yellow and loosely fluffed at other side of culture (Fig. 3).

No. 34D. Orange to dark olive brown pile near inoculum; pale lemon and loosely tangled fluff at other side of culture.

No. 39A. Reddish brown near inoculum; reddish brown pile with numerous short tufts and fluffy cream-color masses at other side of culture (Fig. 3).

No. 39B. Reddish brown near inoculum; a mixture of light orange and dark cream at far side of culture.

No. 39C. Zones of pale, sparse growth alternate with zones of orange pile near inoculum and with zones of cream-color fluffy growth at far side of culture (Fig. 3).

No. 40A. Growth near inoculum mostly pale and scantily fluffy; rest of culture consists of light to dark yellow fluff (Fig. 3).

No. 40B. Sparse and pale near inoculum, with some destruction of agar; rest of culture dark lemon yellow to olive or red brown (Fig. 3).

[6] "A sporophore was removed from a culture with sterile forceps and quickly fastened to the cover of a sterile Petri dish by means of a drop of melted agar. Then the cover was removed from a Petri dish containing lactose gelatine, the cover bearing the sporophore was substituted, and slowly rotated. Then the cover was replaced and as many Petri dishes as desired were inoculated in this way. The plates were kept at room temperature for from four to five days. By that time, the germinating spores appeared as tiny depressions in the surface of the clear gelatine. A circle was drawn around each mycelium, it was examined under the microscope, and if it proved to be monosporous, and if there were no other spores within the circle, it, surrounded by a small square of gelatine, was removed with a sterile spear-shaped needle, placed on a potato-dextrose or malt agar slant, and grown at room temperature."

[7] Numeral refers to parent mycelium—*e.g.*, No. 34A is the first monosporous mycelium secured from No. 34.

No. 40C. Mostly sparse and pale near inoculum; rest of culture raggedly fluffy and pale yellow to dark creamy yellow or light orange.

No. 40F. Zones of sparse and pale growth alternate with zones of short, lemon yellow tufts (Fig. 3).

No. 40K. Most of agar destroyed in immediate vicinity of inoculum; rest of culture consists of light to dark yellow fluff (Fig. 3).

From these descriptions, and from table 4 and figure 3, it will be seen

TABLE 4.—*Growth of representative mycelia of both monosporous and natural origin (Series II)*

Mycelium No.[a]	Age of cultures—days				Diff. between max. and min. on 16th day
	7	11	16	21	
	Average radius—mm.				*mm.*
1.	15	27	49	77	2
3.	2	13	41	69	4
4.	18	33	64	82	4
34.	6	17	41	63	3
34A.	22	39	65	[b]	0
34C.	18	36	67	[b]	8
34D.	22	40	68	[b]	3
34F.	16	28	48	63	4
39.	8	25	56	80	0
39A.	9	27	55	79	2
39C.	12	26	54	74	2
39D.	10	23	38	54	8
40A.	12	32	63	83	2
40B.	13	31	60	81	7
40E.	12	32	69	[b]	2
40F.	13	30	56	76	7
40K.	11	29	62	[b]	1
41.	13	32	63	82	7
43.	6	19	39	62	9
46.	7	23	56	90	2
48.	8	24	51	84	6

[a] Data for each mycelium are based on 2 cultures.

[b] Entire surface of culture overgrown (radius greater than 90 mm.).

that differences between monosporous mycelia are of the same general type and of about the same order of magnitude as those between isolations secured from different sources in the field. Cultural differences are ill-adapted to quantitative expression, and an exact comparison of the extent of variability in the different groups of monosporous mycelia of common descent is, therefore, impracticable; in general, however, it may be said that mycelia having the same immediate ancestry appeared to differ less than did those of more diverse descent. Individual differences were apparent, however, even be-

tween mycelia descended from the same sporophore. When cultured under the same conditions, hyphal-tip transfers from opposite sides of a given monosporous colony were culturally identical.

Twenty-four of the 41 monosporous mycelia secured from No. 40 produced sporophores. From one of these fertile monosporous mycelia (No. 40L) 33 single-spore cultures were made, of which 30 produced sporophores. These third-generation cultures resembled each other very closely, much more so than did those of the preceding generation, but could not be considered identical in appearance. The only other sporophores that appeared in either monosporous or polysporous cultures were developed by a few descendants of Nos. 39 and 50. Some of these were presumptively of single-spore origin, but a few of the supposedly monosporous cultures (including the fertile ones) derived from No. 39 showed striking similarity to polysporous cultures from the same mycelium: In view of the defects of the dilution method (used with spores from this mycelium only) the monosporous origin of these cultures is doubtful.

Polysporous cultures from Nos. 34 and 40 were similar in general appearance to isolations secured in the field, but polysporous cultures from Nos. 39 and 50 displayed none of the gross characteristics of *Polyporus schweinitzii*. The hyphae in the latter two were hyaline, giving cultures on malt agar a dirty grey appearance, and were almost entirely confined to the medium within 1 mm. of the surface of the agar slants. Polysporous cultures from No. 39 produced a few short aerial hyphae that looked like very fine cotton lint sparsely sprinkled on the surface of the agar. Those from No. 50 produced no aerial growth. In other respects these cultures were indistinguishable from one another. After repeated transfers, several of the polysporous cultures from No. 39 developed thin, irregular, dark reddish brown mats on the surface of the agar slants: such cultures invariably produced sporophores.

DISCUSSION

Morphological or cultural characters and more purely physiological qualities are not necessarily correlated (1). Nevertheless, the wide range of variability apparent between the cultures from Springwater, a range almost as great as that found between cultures from widely separated points and unlike hosts throughout the Northern Hemisphere, indicates that damage in this locality cannot now be attributed definitely to infection by a race or strain of special virulence. The planting stock used in the infected areas at Springwater came from nurseries in which seedlings from European nurseries had been transplanted, and other plantings in the immediate neighborhood have been made with stock of European origin, but the large number of different mycelia isolated from the infected trees precludes the possibility of infection having originated entirely by vegetative spread of the organism

from one or a few foci in nursery beds. Fruiting bodies of *Polyporus schweinitzii* have been rare in these plantings until quite recently.[8] It seems unlikely, therefore, that a few original infections by a virulent strain have been spore-propagated, with consequent morphological diversification of the organism, to a sufficient extent to account for the large and varied population of this species now present in these areas. Wean[9] has shown that infection may occur in very young trees, but his studies also indicate rather definitely that parasitic activity of this fungus is favored by certain conditions, such as those at Springwater, which are suboptimum for white pine. Pending the completion of inoculation studies in the field it must accordingly be concluded that the *Polyporus schweinitzii* epiphytotic in this locality probably is attributable to edaphic factors that have permitted extensive infection by native representatives of the species.

The possibilities of future specialization created by the wide variability exhibited by this fungus must not be overlooked. It may be assumed that the majority of the individuals constituting this species are now adapted to a quasi-parasitic life in the basal heartwood of older trees, but extensive infection of younger trees growing under more or less unnatural conditions might conceivably result in the gradual selection of lines best adapted to such existence and a consequent increase in the average virulence of the species to planted trees even on good sites. The likelihood of such a change and the time necessary for it to occur must remain problematical, but the immediate values involved are great enough to justify the development and adoption of preventive and control measures.

Variability of the type described in this paper is by no means peculiar to the Polyporaceae. Recent pathological literature contains numerous references to similar instances of intraspecific variability in widely separated species of fungi. The work of Magie (10) on *Coccomyces hiemalis*, Johnson and Valleau (7) on *Thielaviopsis basicola*, Palmiter (12) on *Venturia inaequalis*, and Hansen and Smith (5) on *Botrytis cinerea* may be cited as random examples. All of these workers observed differences in cultural appearance between isolations, and in some cases also found differences in such characters as growth rate, spore production, size and shape of conidia, pathogenicity, reaction to toxic substances, and reaction to acidity of the medium. None of these differences were correlated with the host or locality from which the isolations were made. Palmiter's (12) statement seems generally applicable to all of the above-mentioned species: "No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures could easily be recognized. . . . *V. inaequalis*

[8] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

[9] Wean, R. E. The parasitism of *Polyporus schweinitzii* on seedling *Pinus strobus*. Unpublished manuscript.

is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.'' Research on such highly heterozygous species can be of maximum usefulness only when the sampling errors inseparable from studies of varied populations have been reduced by the use of a large and representative basis. Small bases may be sufficient in species characterized by slight variability, but in most instances a high degree of variability must be assumed until investigations of numerous isolates have shown the species to be essentially uniform for the character in question. It seems probable that discrepancies between quantitative data secured by competent workers often may be charged directly to the basing of studies on only one or a few individuals from species in which wide variation may exist. For example, Lagerberg and Melin,[10] Robak (13), and Weis and Nielson (16) disagree rather widely as to the optimum pH for growth of *Fomes annosus* (Fr.) Cke. in artificial culture. This disagreement may be the result of differences in experimental conditions, but the observations of Palmiter (12) and the present writer suggest that it is caused by differences between the individual mycelia used in the various studies.

The term ''strain'' often is employed by pathologists to designate a single original isolation and its subcultures (with the exception of saltants, etc.). Its use in this particular connection is unfortunate, since it frequently leads the reader to an erroneous conception of the relationships involved, even when it does not indicate a misunderstanding on the part of the user. ''Strain'' suggests a racial grouping[11] of genotypically distinct individuals that are homozygous for one or more physiologic or cultural characters serving to distinguish them from other members of the species. In many instances the term is, of course, correctly used, but it is not applicable to any one of the different mycelia of *Polyporus schweinitzii* herein described. Genetic irregularities and the frequent occurrence of asexual reproduction obscure the individualization of fungi, but in this group, as in any other group of organisms, individuals are the concrete units into which such abstract conceptions as strains and species are ultimately resolvable. The situation is made more complex but is not fundamentally altered by such anomalous phenomena as mixochimaeras (heterocaryotic cells), ''discontinuous variations'', and physical discontinuity of individual genotypes. For practical purposes, separated fragments of an organism or new colonies

[10] Not seen by the present writer; *fide* Weis and Nielson (16).

[11] ''When the isolates are compared on various culture media and under different environmental conditions they fall into a number of groups each of which contains isolates which are alike in their cultural characters and cultural behaviour. Each of these groups constitutes a Strain or Race, and equates with Lotsy's 'Jordanon'. . . . Cultural races may be equated with biological races in the more strict sense.'' Brierley (1).

that have originated from accessory spores of the same genotype can be accorded no greater claim to separate individual ranking in Nature than *in vitro*.[12] Brierley's isolates, then, do not necessarily represent different individuals in all cases, although for the sake of convenience they must often be considered to do so. ''Mycelium'' has been used throughout the present paper in a sense that the writer considers equivalent to ''clone''. ''Genotype'' or, less accurately, ''individual'', might be similarly used. Choice of terms must frequently depend, as in the present instance, on convenience of expression and on the extent to which relationships within the species in question are known. Whatever the terminology, the essential fact remains that some species of fungi, of which *Fomes pinicola* (11) and *Polyporus schweinitzii* may be cited as examples among the Polyporaceae, are made up of individuals that differ from one another in many respects, and that investigations of such species must, therefore, include a sufficient number of individuals to permit an approximate determination of the specific average and range of variability.

SUMMARY

Cultural studies of 50 different mycelia of *Polyporus schweinitzii*, secured from various coniferous hosts and widely separated points throughout the Northern Hemisphere, show that this species is made up of individuals that differ rather widely from one another in appearance in culture, production of sporophores, growth rate on nutrient agar, reaction to acidity, and apparently also in ability to cause decay of wood. Individual differences also were found between monosporous mycelia secured from spores produced by pure cultures of the fungus. There was no evidence of the occurrence of local or host-specialized strains within this species, and it therefore seems probable that the serious damage which the fungus is causing to planted white pine near Springwater, N. Y., is attributable to site conditions rather than to any special virulence on the part of the fungus in these plantings. The differences shown to exist between individuals of this species are comparable to those demonstrated by Mounce (11) for *Fomes pinicola*, and indicate that research on such variable species can produce generally applicable results only when a large number of individuals are studied.

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STRUCTURE OF THE INTERKINETIC NUCLEUS IN THE SCALE EPIDERMIS OF ALLIUM CEPA

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Although the interkinetic nucleus has been intensively investigated for more than half a century, there is at present little agreement among cytologists as to the properties of the nuclear membrane or the structure of the chromatic elements. The fact that the genes can be assigned definite loci in particular linkage groups has raised the question as to the possible persistance of such gene-strings throughout the interkinetic phases, and has focussed attention on such structural details in the resting nucleus which might serve as the physical basis of such linkage groups. It is interesting that some of the earlier investigators described individual coiled threads in such nuclei, although they were unaware of the bearing of their findings upon any possible theory of the mechanism of heredity. Recent advances in cytological technique, micro-manipulation, etc. have made it probable that a re-investigation of the resting nucleus would reveal new facts which might have some bearing on the genetical problems involved.

MATERIAL AND METHODS

In the experiments designed for the study of the nuclear membrane, rectangular strips of epidermis from the onion-scale were mounted on a slide and flooded with the various reagents. Then, with a sharp razor the strips were cut across the longitudinal axis of the cells. Thus the nuclei were either extruded into the mounting medium or the various reagents had immediate access to the nuclei in the cut cells. The nuclei in similar pieces of tissue mounted in a hanging drops against the surface of cover slips were dissected with a CHAMBERS' micro-manipulator, while the uncut specimens were placed directly into the various fixatives tested.

In order to investigate the osmotic properties of the nuclear membrane, extruded nuclei were mounted in solutions of M/4 glycerine and M/4 sucrose, isotonic with the cell sap, and in distilled water. The effects of the presence and absence of calcium ions on the persistence of the membranes and the internal structure of the nuclei were observed in isotonic solutions of glycerine, sucrose and once distilled water, all containing traces of either $CaCl_2$, C_2HO_4Na or $C_2H_2O_4$. These solutions were used over a wide pH range, the hydrogen-ion concentration being measured colorimetrically. The nuclei were also extruded into M/8 $CaCl_2$ and M/8 KCl in order to observe their responses to calcium and potassium in high concentrations.

The well known cytological fixatives were used, such as NAVASCHIN, weak FLEMMING, BOUIN, potassium dichromate, nickel/copper bichromate-propionate, chromic-formic-acet-

aldehyde, ERLIKI's mixture modified with ethylene diamine and some new mixtures, such as aluminum/uranyl nitrate-acetaldehyde-alcohol, calcium chloride-hydrochloric acid and tribasic sodium phosphate-formaldehyde-sucrose. The fixatives were selected to note the effects of those which are extremely acid and of those which are decidedly basic.

Fixation in all cases was for a period of forty-eight hours, but frequent examinations of the specimens were made during the process. The specimens were then washed and stained in haematoxylin, Feulgen nucleal stain, aceto-carmine, or with Cloth Red B dissolved in HCl. Cloth Red B differs but slightly in chemical composition and in staining reaction from Orseillin B. B. reported by COHEN and DOAK (5). A few drops of M/8 HCl half-saturated with Cloth Red B added to 10 c.c. of M/10 $CaCl_2$ stains the nucleus sharply and the finer details of material fixed in this mixture can readily be photographed.

RESULTS

Many investigators have indicated that the nuclear membrane is not a simple structure. SCHWARZ (22), ROBYNS (16), STRÜGGER (24, 25) have pictured the membrane as double, while SCARTH (18) and KLINGSTEDT (11) reported that the outer membrane is cytoplasmic in origin. In addition, SCARTH noted differences in the osmotic properties of the two membranes.

When nuclei of onion epidermis are freed into distilled water, exposed to air to reach a pH of 6.5, they may react in one of two ways. They either swell in such a manner that no details of their structure become visible, or, more frequently, they swell so that two distinct membranes separate. At first, only small areas of the outer membrane form what appears to be small blisters but, later, as more of the outer membrane becomes involved, these blisters coalesce into a common vesicle, encompassing the nucleus with its inner membrane. The nucleus then shrinks, while the outer membrane, now clearly visible and presumably of cytoplasmic origin, expands, the two membranes becoming well separated (Figs. 1, 2, 3, 4). These changes occur so rapidly that it is a matter of a few minutes before the nucleus shrivels, while the outer membrane becomes progressively thinner, finally bursting when its contents have attained a volume approximately twenty-four times greater than that of the original nucleus. When nuclei are placed in M/4 glycerine, an isotonic solution, their reactions are similar to those placed in distilled water but much slower, the swelling occuring apparently as the glycerine penetrates. As the glycerine slowly penetrates the nuclei, it raises their osmotic value while their surrounding medium becomes hypotonic. In M/4 sucrose no swelling occurs and the nuclear membranes do not separate, the sucrose apparently not penetrating at all (Fig. 5). In these experiments the hydrogen-ion concentration did not vary significantly. The solutions, although unbuffered except for slight impurities in the glycerine, remained at pH 6.4—6.5. In order to note the effect of free calcium on the persistence of the nuclear membranes and their reactions, the experiments were repeated with a trace of C_2HO_4Na added to the reagents to remove traces of calcium. When nuclei are placed in distilled water containing a trace of C_2HO_4Na, at pH 9.0, the membranes separate, but in M/4 glycerine with a similar addition, the pH now being 6.4, no such separation occurs. In M/4 sucrose with a trace of C_2HO_4Na, at pH 8.0—9.0, the nuclei swell and burst at one side, out of which the liquified

IRREGULAR PAGINATION

nuclear contents then flow. In order to vary the pH a sufficient quantity of $C_2H_2O_4$ was added to bring to pH 6.4 the mixture of M/4 sucrose and C_2HO_4Na. Thus the effects of the oxalate ion in sucrose could be compared at the same pH with its effects in glycerine. In such a mixture the nuclei do not swell, but lose all indications of internal structure, and moreover the membranes do not separate. If the M/4 sucrose solution containing the mixture of C_2HO_4Na and $C_2H_2O_4$ at pH 6.4 is replaced by one containing only C_2HO_4Na at pH 9.0, the nuclei swell and burst as they did in the previously cited case.

To differentiate further the responses shown by the nuclei to changes in the hydrogen-ion concentration, $C_2H_2O_4$ was used in place of C_2HO_4Na. When a trace of $C_2H_2O_4$ is added to distilled water, M/4 glycerine and M/4 sucrose, bringing all to pH 3.0—4.0, the nuclear membranes do not separate, while in distilled water with $C_2H_2O_4$ at pH 4.6, the isoëlectric point of many proteins in the cell, the membranes separate but slightly on one side of the nucleus. In M/4 glycerine with $C_2H_2O_4$ at pH 3.0—4.0, the nuclei behave in a peculiar fashion. Intranuclear alveoles arise and break through the nuclear membranes, the nuclei thereby becoming irregular and ragged. The membranes do not separate in the nuclei liberated into distilled water, M/4 glycerine or M/4 sucrose to which HCl had been added to bring their pH to 3.0.

In the presence of $CaCl_2$ there is a significant difference in the behavior of nuclei placed respectively in distilled water, in M/4 glycerine and in M/4 sucrose, all at pH 6.4—6.6, as contrasted with their behavior when immersed in the same solutions without traces of calcium. The membranes of those nuclei immersed in the M/4 sucrose with an added trace of $CaCl_2$ do not separate. Moreover, the nuclei here do not noticeably differ from those placed in M/4 sucrose with added traces of HCl, bringing the pH to 3.0 (Fig. 6). At first, the nuclei in distilled water and in M/4 glycerine, pH 6.4, with calcium appear to be no different than those freed into the calcium-containing sucrose, but in a short time the membranes separate and the nuclei shrink, although they maintain their internal structure (Fig. 4). The outer membranes of the nuclei so treated are much thicker, possibly due to the adhering cytoplasm coagulated by the calcium, than those nuclei in distilled water with a sufficient quantity of the oxalate ion to insure the absence of calcium, provided the acidity is less than pH 4.6.

In order to observe the effects of incidental changes in the hydrogen-ion concentration in the presence of free calcium, nuclei were placed respectively in distilled water, in M/4 glycerine and in M/4 sucrose to which a trace of a mixture of $CaCl_2$ and HCl had been added to pH 3.0. The behavior of the nuclei here did not differ essentially from those freed into the similar liquids containing only HCl. On the other hand, nuclei extruded into a saturated aqueous solution of $Ca(OH)_2$, having a pH of 8.4, may react in one of two ways. They either swell in such a way that no details of their structure become visible or, less frequently, they swell so that two distinct membranes become separated from each other. The outer membranes around the latter nuclei are much thinner than those separating from the nuclei in distilled water to which traces of $CaCl_2$ (pH 6.4—6.6) had been added. This suggests that the coagulative action of calcium on the

cytoplasm is definitely influenced by the decrease in the hydrogen-ion concentration. In order that some insight might be gained as to the action of the hydroxyl ion in high concentrations, nuclei were freed in very dilute NH_4OH (one drop of concentrated ammonia water to fifty c. c. of distilled water) having a pH 9.8. Here the nuclei were constant in their response, merely swelling without showing any separation of the membranes.

Since the presence or absence of calcium in certain of the described mixtures of distilled water, glycerine and sucrose produced such marked reactions on the part of the extruded nuclei, it was considered to be of sufficient importance to follow their behavior in solutions containing large amounts of calcium. When nuclei were placed in M/8 $CaCl_2$, pH 6.8, they reacted in one of two ways. More frequently they became reticulated from the outset and then solated, thus apparently losing all internal structure. The Brownian movement was very vigorous during the solation of the reticulum. The nuclei were rigid and appreciable amounts of coagulated cytoplasm adhered to their unseparated membranes (Figs. 12, 13). Less frequently their internal structure was preserved generally in one of two ways, the chromonemata being either accentuated or changed into a reticulum. The membranes slowly separated and the nuclei gradually shrank to a small size within the outer membrane, which attained a volume several times greater than the original nucleus (Fig. 14).

By extruding nuclei into a M/8 KCl solution an opportunity was afforded to follow the reactions of nuclei in high concentrations of potassium and where the concentration of calcium, was too dilute to produce any characteristic response on the part of the nuclei. The nuclei were found to respond in one of three ways. First, they may become reticulated with the two membranes separating. The nuclei, still delimited by their inner membrane, shrink within the vesicle bounded by the outer membrane (Figs. 8, 11, 15). The separation of the membranes is rarely completed and the entire structure collapses at the end of five hours. Secondly, the outer membrane may lift from the nuclear surface, but soon collapses, the nuclei remaining reticulated without any further change (Figs. 9, 10). Finally, the nuclei may become reticulated without any separation of the membranes occuring. When uncut strips of onion epidermis are mounted in M/8 KCl, cytoplasmic streaming continued without any apparent change in the nuclei within the intact cells. Evidently at approximate isotonic concentrations KCl does not materially affect the cells. In M/5 KCl, however, the cells are plasmolyzed within two minutes and the nuclei become reticulated, indicating a disruption of the colloidal phases in the nuclei. When such strips of epidermis are fixed and stained in aceto-carmine, it is possible to arrange a series of nuclei with reticula of many interstices to reticula with but few interstices (Figs. 24, 25, 26, 27).

A careful examination of the nuclei in healthy onion epidermis does not lend support to the view that the interkinetic chromatin is distributed in a reticulum, showing it to be present rather as entangled chromonemata. From a mere cursory examination, one could readily gain the impression that the chromatin is disposed as a reticulum in the resting nucleus. This apparent reticulum

is however due in no small measure to the fact that the chromonemata lie in the lower limits of microscopic visibility and the points where they pass over one another can accordingly be scarcely distinguished from points of fusion. In addition to the fact that the gyres of a chromonema are only partly visible at one focal level, the chromonemata may dip sharply into the nucleus and be partly superimposed upon each other, thus increasing the difficulty of tracing their entire length. As early as 1885, RABL (14) was unable to decide whether he saw in the interkinetic nucleus a real network or independent threads which merely passed over one another. In most onion nuclei it is possible to single out a chromonema which lies at a high focal level for a greater part of its length and by gradual adjustments in the focus to follow the coiling, and even changes in the direction of coiling in the chromonema (Figs. 6, 20). The chromonematic structure is accentuated in nuclei freed in M/4 sucrose to which a trace of HCl, yielding pH 3.0, or of $CaCl_2$, pH 6.4—6.6, had been added (Fig. 6); and in nuclei placed in M/8 $CaCl_2$ with HCl, the chromonemata are shown most clearly. The chromonematic structure of the same nuclei can be made to disappear, the chromatin apparently disssolving, by replacing the solutions with M/4 sucrose containing the above described amount of C_2HO_4Na at 8.0—9.0. When HCl is then added, structure reappears, but this resultant structure is clearly a reticulum. That the disruption of the true chromonematic structure in the above cited cases was not due to the incidental changes in the hydrogen-ion concentration is shown by the fact that nuclei which had been placed previously in a saturated aqueous solution of $Ca(OH)_2$ and which appeared to be optically empty would revert to their original chromonematic structure when HCl was added. The effect of calcium removal from nuclei placed in solutions of high acidity is most clearly demonstrated by the nuclei placed in M/4 sucrose to which has been added $C_2H_2O_4$ at pH 3.0—4.0. The chromonematic structure is visibly altered in a series of changes suggestive of BĚLAŘ's (3) description of an "Entmischung". The formation of the reticulum in the nuclei so treated follows directly upon the collapse of the chromonemata and the accompanying changes. The formation of the reticulum in nuclei first treated in M/4 glycerine containing C_2HO_4Na, pH 6.4, so that the chromatin is apparently dissolved, is due to a precipitation when the glycerine mixture is replaced with one containing HCl. The reticulum thus formed bears no resemblance to the original structure present before the solation of the chromatin in the presence of the oxalate ion. When the chromatin disappears in this case, it must be assumed to have dissolved, for the structure which reappears with the change in the hydrogen-ion concentration differs markedly from that originally present. However, the chromatin in what are apparently empty nuclei is not necessarily dissolved, for in the case of nuclei placed in $Ca(OH)_2$, the chromatin can be made to reappear in its original from. Thus the description of nuclei as optically empty is of no particular significance unless it be accompanied by evidence showing that the nuclei under discussion really have no internal structure.

Although the nucleoli remain visible in the nuclei immersed in distilled water containing a trace of C_2HO_4Na, they disappear in those nuclei placed in M/4 glycerine to which had been added a comparable amount of C_2HO_4Na or

$C_2H_2O_4$. In the nuclei extruded into very dilute NH_4OH, the nucleoli disappear as they in $Ca(OH)_2$; when treated with the latter reagent, however, they can be made to reappear with acidification, thus indicating a marked difference in the action of the two hydroxides. Usually two nucleoli are visible at different focal levels in the living interkinetic nucleus. In nuclei treated with M/4 sucrose containing a mixture of C_2HO_4Na and $C_2H_2O_4$, at pH 6.4, these nucleoli fuse, and the resultant fusion nucleolus is of irregular outline, bearing no resemblance to the individual participating nucleoli.

In fixed and stained material the chromonematic structure of the nucleus can be best observed in strips of onion epidermis fixed overnight in a mixture of M/10 $CaCl_2$ (10 c.c.) and a few drops of M/8 HCl half-saturated with Cloth Red B. The nuclei are delicately stained and a permanent mount can be made easily. Acid salts of metals such as aluminum, as well as uranyl nitrate, when combined with acetaldehyde and alcohol, give fairly reliable fixation images. With such fixations, however, the chromonemata show a tendency to collapse in part, producing incidentally artificial chromatin nucleoli (Figs. 17, 18). Since they may collapse through only a small portion of their length, such artifacts could readily be mistaken for plasmosomes were it not for the fact they stain with the Feulgen technique, and are thus unlike true plasmosomes. Moreover, in the living nucleus such chromatin nucleoli are not visible, whereas the real plasmosomes, having an index of refraction different from the chromatin, can readily be seen. While no evidence is presented here that contradicts the reality of prochromosomes, it is necessary to demonstrate that the chromonemata have not collapsed into such artificial chromo-centers (HEITZ 8, 9).

As a rule most of the acid fixatives tested preserved the nuclei with reticula (Figs. 19, 20) but, in a few instances, the true chromonematic structure was retained in some nuclei while in others the chromatin was precipitated in many intermediate forms. Such was the case in three of the most widely used cytological fixatives, BOUIN, NAVASCHIN and weak FLEMMING. These fixatives, containing large amounts of acetic acid, usually preserve the nuclei in material of several cell layers in thickness as hollow structures with a halo between the nucleoli and the reticulum. That such reticula and haloes are not due entirely to fixation, but in part to the methods employed in paraffin infiltration, is indicated by a comparison of onion epidermis and of root tips fixed in these fluids. While the collapse of the chromonemata is one of the most important factors contributing to the formation of the reticulum, the fact should not be overlooked that acetic and chromic acids have already been reported to produce artificial reticula (BALBIANI 1, NEBEL 12). The lymph of the nuclei in onion epidermis fixed in BOUIN, NAVASCHIN and weak FLEMMING solutions was better preserved than in those nuclei examined in the root tips. In addition, there was no appreciable halo between the reticulum and the nucleoli. ZIRKLE (30) has already stressed the fact that the fixation image is greatly influenced by the relative rates of penetration of the various components of a fixative. In smears and in material of a few cell layers in thickness, such as onion epidermis, there is of course no such difference in the fixation image caused by this factor.

The fixatives which are most commonly used to preserve mitochondria if made more alkaline, preserve the nuclei as if devoid of chromatin. In some cases there is an actual dissolving out of the chromatin, as when nuclei are treated with a mixture of M/8 tribasic sodium phosphate-4 M/3 formaldehyde-M/4 sucrose (Fig. 23). Such nuclei stain diffusely with the Feulgen technique, acidulated Cloth Red B or carmine, while with haematoxylin they stain with great difficulty, if at all. If strips of onion epidermis have had a prior treatment with dilute NH_4OH and then are fixed and stained in aceto-carmine, it is possible to arrange a series of nuclei indicative of the various stages in the dissolution of the chromatin and other nuclear structures (Figs. 28, 29, 30, 31). On the other hand, nuclei fixed in chromic sulphate-formaldehyde, a reported basic fixative, show a fine preservation of the chromonemata and the maintenance of the structural relationships of the parts of the nucleus which can not be successfully stained with haematoxylin, but stain very easily with acidulated Cloth Red B. Nuclei fixed in Erliki's mixture modified with ethylene diamine and in potassium dichromate alone reacted similarly in being hard to stain with haematoxylin, and showed several stages in the disorganization of the chromatin structure. In several instances, cytoplasmic alveoles may indent nuclei fixed in potassium dichromate, so that after dehydration the nuclei are distorted (Fig. 22).

The primary purpose of micro-dissection was to attempt to pull the chromonemata out of the nucleus and count them. The nuclei from onion epidermis were freed in the usual manner against the surface of the cover slip in a hanging drop of M/4 sucrose with a trace of $CaCl_2$, the cover slip serving as the roof to the moist chamber of the micro-manipulator. Such nuclei can be pushed about with the point of the micro-needle since they are quite rigid, but if they are punctured, the nuclear lymph, though rather viscous, then flows out through the puncture and the structure collapses. The collapsed nuclei prove to be surprisingly elastic when seized by both needles and stretched. When the nuclei are being stretched, the chromonemata are deformed and, before breaking into small segments, become alligned parallel to the direction of pull. If the chromonemata are pulled out of the nucleus, which thereupon collapses, they become immediately rounded up into shapeless blobs at the end of the micro-needle or on the surface of the cover slip.

The presence of cytoplasmic streaming in uncut cells at the end of the experiment was used as a check of the original healthiness of tissue. In the literature there are several reports on the effect of wounding being transmitted to nuclei far removed from the involved area. In onion epidermis, cells next to those which had been cut open were not affected and cytoplasmic streaming continued in them without any alteration in the appearance of the nuclei.

DISCUSSION

Although the existence of a nuclear membrane was suggested by Robert Brown as early 1833, it was noted by few other workers prior to 1884. In fact many believed that there was no such membrane and the entire question became highly controversial. Tischler (26) and Heidenhain (7) have both reviewed adequately this earlier literature. It is of interest that Schwarz (22) reported the nuclear membrane to be osmotic. He subjected onion nuclei to the action of distilled water and observed that blisters were produced on their surfaces. These he termed "Randvakuolen" and noted that the blisters coalesced to form a single, large peripheral vacuole which enclosed the entire nucleus, thus indicating that the nucleus had two distinct membranes. Furthermore, Scarth (18) and Klingstedt (11) confirmed the existence of these two membranes. Scarth explains the observed differences in the nuclear membranes of his material (cockroach oöcytes) as due to their osmotic differences.

In general, the reaction of nuclei placed in distilled water or in M/4 glycerine indicates that they are surrounded by two membranes, the outer of which, as observed by Schwarz, is truly osmotic. Although the inner membrane does not seem to be semi-permeable, it is quite possible that it is semi-permeable in the living cell and that this property is lost as soon as it is taken out of the cell. The outer membrane is very thin and apparently there is no cytoplasm adhering to it. In addition, it does not persist for as long a time in distilled water alone as it does when the nucleus is extruded into distilled water containing a trace of calcium (Fig. 4). When the outer membranes separate in those nuclei placed in distilled water or in M/4 glycerine with traces of $CaCl_2$, they are much thicker, probably due to the coagulated cytoplasm adhering to them, than those membranes which separate in solutions containing an excess of oxalate ions or in M/8 KCl where the absence of calcium is assured. It is well known that calcium plays an important role in membrane formation, and its absence from solutions in which nuclei are freed might permit the outer membranes to be dissolved or be inhibited from separating. The outer membranes of nuclei placed in distilled water with a trace of C_2HO_4Na separate before there any action of the oxalate ion is noted. In cases where the separation of the membranes is normally slow, the outer membranes may be dissolved before they separate. This is apparently what occurs when nuclei are subjected to the action oft he oxalate ions in M/4 glycerine and M/4 sucrose.

The coagulative action of calcium is probably influenced by the changes in the hydrogen-ion concentration. The membranes which separate from nuclei placed in $Ca(OH)_2$ at pH 8.4 are much thinner and obviously lack the coagulated cytoplasm seen with those thicker membranes of nuclei placed in distilled water or in M/4 glycerine with added traces of $CaCl_2$ at pH 6.4—6.6.

Optical analysis of protoplasm is inadequate to determine whether or not structure or a definite pattern exists. Although the investigations with polarized light may reveal a fundamental arrangement of submicroscopic particles in protoplasm (Schmidt 19, 20), the usual optical systems employed in microscopy are not able to show these structures. For example, nuclei freed in $Ca(OH)_2$ appear to be optically empty but are really not so as is proven by their behavior when treated with dilute HCl. When thus acidified, the nuclei reveal a chromonematic structure which is essentially their form in the living cell. Changes from optically homogeneous structure in nuclei to those which show visible detail are well

known in the living cell. WEBER (28) found that the nuclei in the guard cells of the stomata underwent reversible changes depending upon stomatal activity.

The view that the chromatin does not differ in its arrangement in the interkinetic nucleus from that of the chromosomes is rapidly gaining ground, and is in harmony with the results of several modern re-investigations of the mitotic cycle. If the chromonemata persist through the interkinetic phases in the nuclei of higher plants, we can readily see how the linear order of the genes is preserved in the linkage groups. In well fixed nuclei of onion root tips, the chromosomes are visibly coiled in the earliest prophase stages. The chromonematic structure of the interkinetic nucleus can be demonstrated best in thin strips of onion epidermis and in well fixed smear preparations (NEBEL 13, SAX and SAX 17). SAX and SAX (17), by prior treatment of the resting nuclei in *Tradescantia* microspores with ammonia-alcohol, were able to preserve the chromonemata even with acetic acid fixation.

The formation of the reticulum in the interkinetic nucleus can readily be interpreted as to the collapse of the chromonemata which are appreciably coiled at this stage. Nuclei behave in this manner when treated in M/4 sucrose containing traces of $C_2H_2O_4$. A comparative study of nuclei in the same strip of epidermis of from different strips fixed in the same fluid reveals a series of fixation images from well preserved nuclei with chromonemata to badly preserved nuclei with reticula. The cause for such variations in the specimens may be due to local differences in the vacuolar contents of the different cells. Linin was not observed in either living or fixed nuclei. Although no attachments were seen between the chromonemata and the nucleoli in either living or fixed nuclei or those fixed in the copper or nickel salts of chromic and propionic acids, there is no evidence here that disproves the possibility for such an attachment.

The observations and results reported here may not be generally applicable to animal nuclei or even to the nuclei of the lower plants. The formation of vesicular nuclei in which the chromosomes retain their pellicle into interkinesis has been demonstrated by WENRICH (29) and RICHARDS (15). In addition, the processes extending from the telophasic chromosomes in certain animal nuclei have been shown to be real structures, and these structures coalesce to form what is apparently a true reticulum.

SUMMARY

1. Two membranes invest the extruded, interkinetic nucleus of *Allium Cepa*. In distilled water or in M/4 glycerine, an outer membrane, probably cytoplasmic in origin, separates from an inner one which remains intimately associated with the nuclear contents. The rate at which the membranes separate is related to the rate in change of the osmotic values within the nuclei. The behavior of the outer membrane indicates that it possesses semi-permeable properties which the inner one lacks under the same experimental conditions.

2. The outer membrane is not preserved in the presence of oxalate ions except when nuclei are placed in distilled water containing C_2HO_4Na at pH 9.0. Here the outer membrane is preserved temporarily since the change in the osmotic value of the nucleus is faster than the dissolving action of the oxalate ion.

3. The outer membrane is preserved by calcium ions over a wide pH range notwithstanding the decrease in the coagulative properties of calcium ions in alkaline media.

4. Independent, coiled chromonemata were observed in living, in moribund and in fixed nuclei. Through micro-dissection the chromonemata can be pulled out of the nucleus, which thereupon collapses.

5. When nuclei are placed in distilled water, in M/4 glycerine and in M/4 sucrose, all of which contained C_2HO_4Na, their chromonematic structure disappears, the chromatin apparently dissolving. If the same nuclei are then treated with dilute HCl, the chromatic structure reappears as a reticulum.

6. When nuclei are placed in $Ca(OH)_2$ at pH 8.4, their membranes are preserved but their chromonematic structure is rendered optically homogeneous. Treatment with dilute HCl causes the same nuclei to return to their original chromonematic structure.

7. The results obtained by the use of various fixatives indicate that the chromonemata and the nuclear membranes are preserved when the fixation image is conditioned by either hydrogen or calcium.

8. The reticulum, reported in the interkinetic nucleus, does not occur in the living nuclei of the epidermal cells of *Allium Cepa*, but originates through the collapse and fusion of the chromonemata caused by either experimental injury or by certain types of fixation.

9. Artificial chromatin nucleoli were produced in certain nuclei by fixation when the coiled chromonemata partially collapsed.

The author is indebted to Professor CONWAY ZIRKLE who assisted him in this investigation, and also to Doctor KENNETH DAVIS DOAK and Mr. VERNON DONEY who assisted him in preparing the photographic illustrations.

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DESCRIPTION OF THE PLATES VI AND VII

Figures 1—23 magnified 1150 diameters, figures 24—31 magnified 1400 diameters.

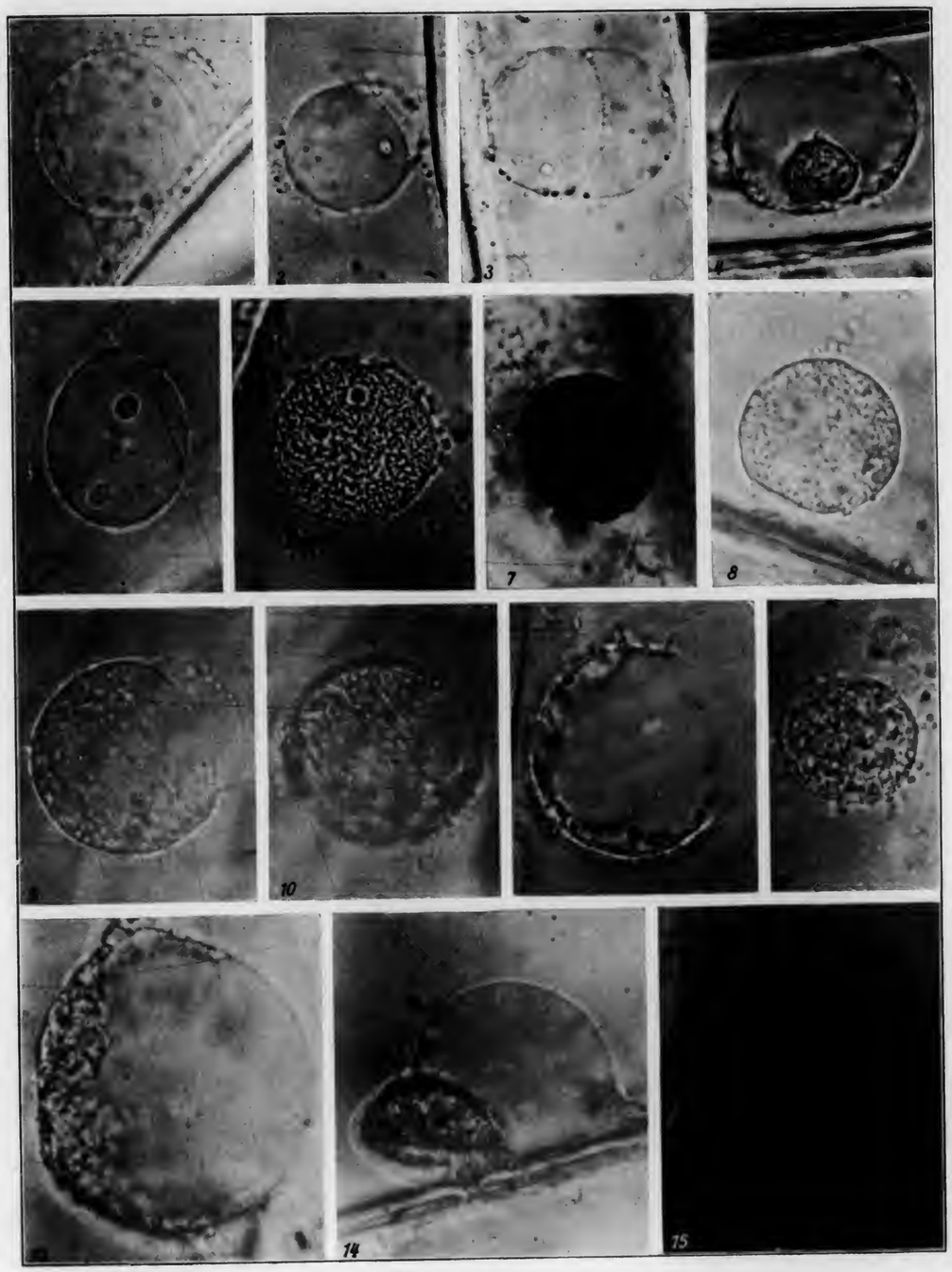
(Plates reduced to three—fourths of original size)

I

Fig. 1. Nucleus extruded into distilled water containing traces of C_2HO_4Na, pH 9.0, showing the separation of the two nuclear membranes and the absence of nuclear structure.
Fig. 2. Nucleus extruded into M/4 glycerine, pH 6.4, showing the separation of the two membranes and the absence of nuclear structure except for the nucleoli.
Fig. 3. Nucleus in the same solution as above. Later stage in another nucleus.
Fig. 4. Nucleus freed into distilled water containing traces of $CaCl_2$, at pH 6.4—6.6, showing the coagulated cytoplasm around the outer membrane and the maintenance of the internal structure in the shrunken nucleus.
Fig. 5. Nucleus liberated into M/4 sucrose, pH 6.4, showing no apparent structure except for the vacuolate nucleoli.
Fig. 6. Nucleus extruded into M/4 sucrose containing traces of $CaCl_2$, pH 6.4—6.6, showing an accentuated chromonematic structure.
Fig. 7. Nucleus fixed in $CaCl_2$-HCl and stained with Cloth Red B. The chromonemata are well preserved.
Fig. 8. Nucleus extruded into M/8 KCl, approximately at neutrality, illustrating the solation of the reticulum.
Fig. 9. Nucleus freed into M/8 KCl, showing the separation of the two nuclear membranes and the solation of the reticulum.
Fig. 10. Same nucleus in Fig. 9, but fifteen minutes later, showing the collapse of the outer membrane.
Fig. 11. Later stage of nucleus extruded into M/8 KCl, illustrating the course of solation. The outer membrane is very thin in comparison to the unseparated membranes.
Fig. 12. Nucleus liberated in M/8 $CaCl_2$, at approximate neutrality, illustrating a reticulum.
Fig. 13. Nucleus extruded into M/8 $CaCl_2$, showing an advanced stage in the solation of the reticulum. Note the thickness of the unseparated membranes as compared with those that have separated in Figs. 1, 2, 3.
Fig. 14. Nucleus freed into M/8 $CaCl_2$, showing the separation of the two nuclear membranes and the maintenance of the nuclear structure (slightly out of focus).
Fig. 15. A late stage in the solation of a nucleus extruded into M/8 KCl.

II

Fig. 16. Nucleus fixed in $CaCl_2$-HCl and stained with Cloth Red B, showing the chromonemata.
Fig. 17. Nucleus fixed in uranyl nitrate-acetaldehyde-alcohol and stained with haematoxylin, showing the artificial chromatin nucleoli at the periphery produced by fixation.
Fig. 18. Nucleus treated the same as in Fig. 17, showing the abundance of the artificially induced "chromatin nucleoli".
Fig. 19. Nucleus fixed in chromic-formic-acetaldehyde and stained with haematoxylin, showing the reticulum in which traces of the chromonematic structure is preserved.
Fig. 20. Nucleus fixed in $CaCl_2$-HCl and stained with Cloth Red B, illustrating well preserved chromonemata.
Fig. 21. Nucleus preserved in chromic-formic-acetaldehyde and stained with haematoxylin, showing a reticulum.
Fig. 22. Nucleus fixed in potassium dichromate and stained with acidulated Cloth Red B. The greater part of the fixed nucleus is preserved nuclear lymph., the chromatin being dissolved.
Fig. 23. Nucleus fixed in tribasic sodium phosphate-formaldehyde-sucrose and stained with acidulated Cloth Red B, illustrating a swollen nucleus of the basic type in which the chromatin is not preserved.
Figs. 24—27. Nuclei treated in M/5 KCl, fixed and stained with aceto-carmine, showing the various degrees of reticulation.
Figs. 28—31. Nuclei treated with dilute ammonium hydroxide, then fixed and stained with aceto-carmine, showing various stages of disorganization.



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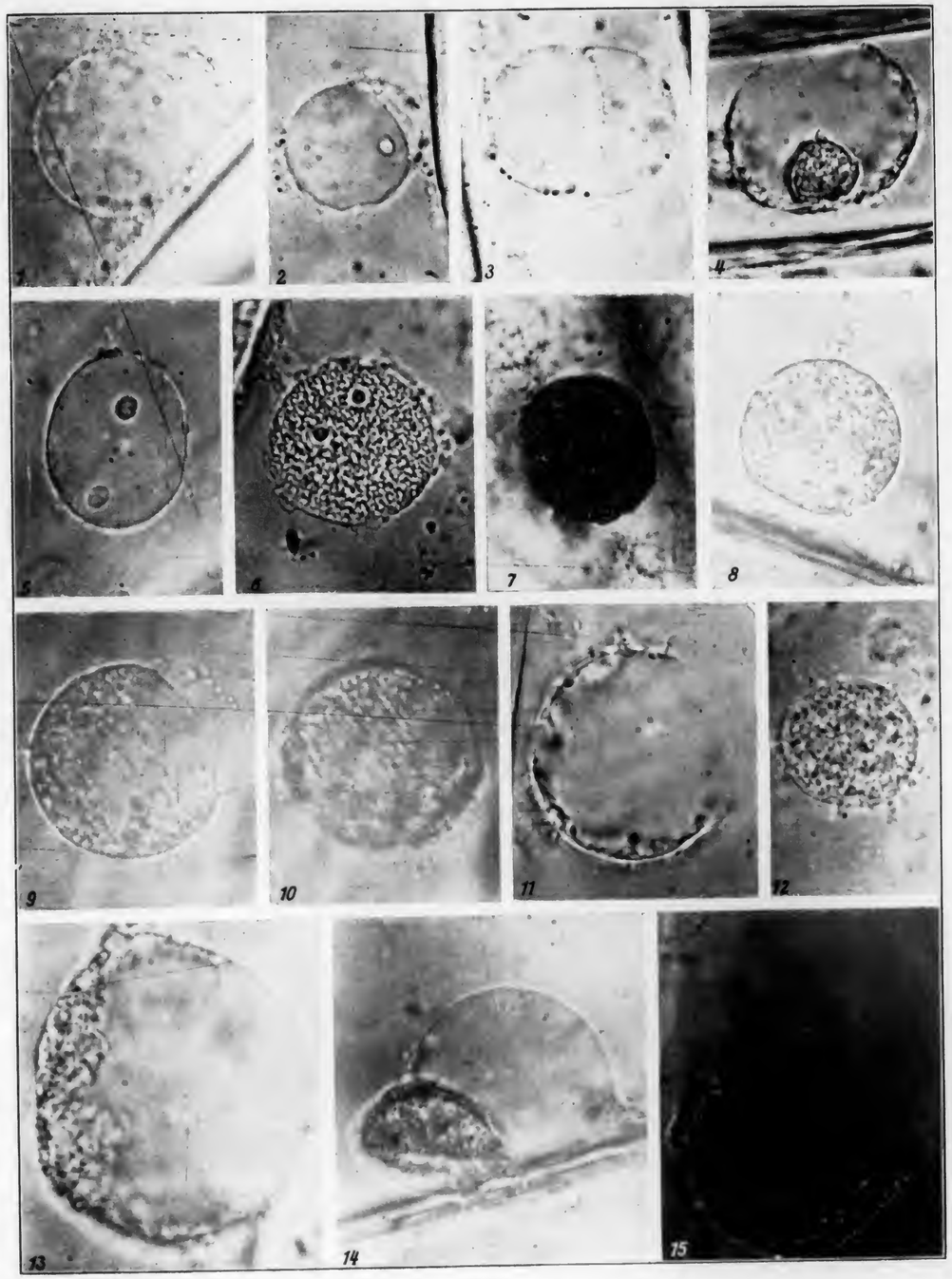

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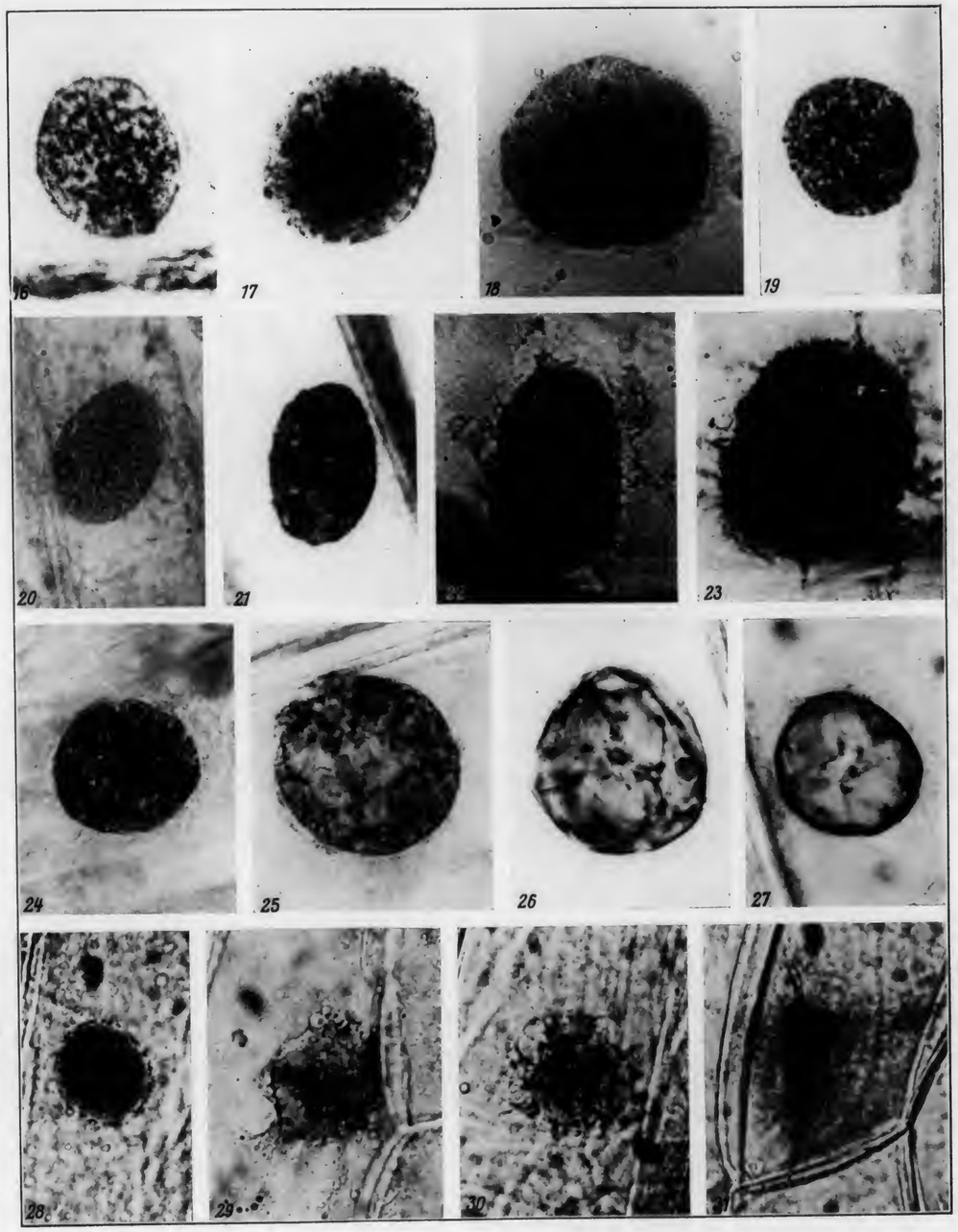

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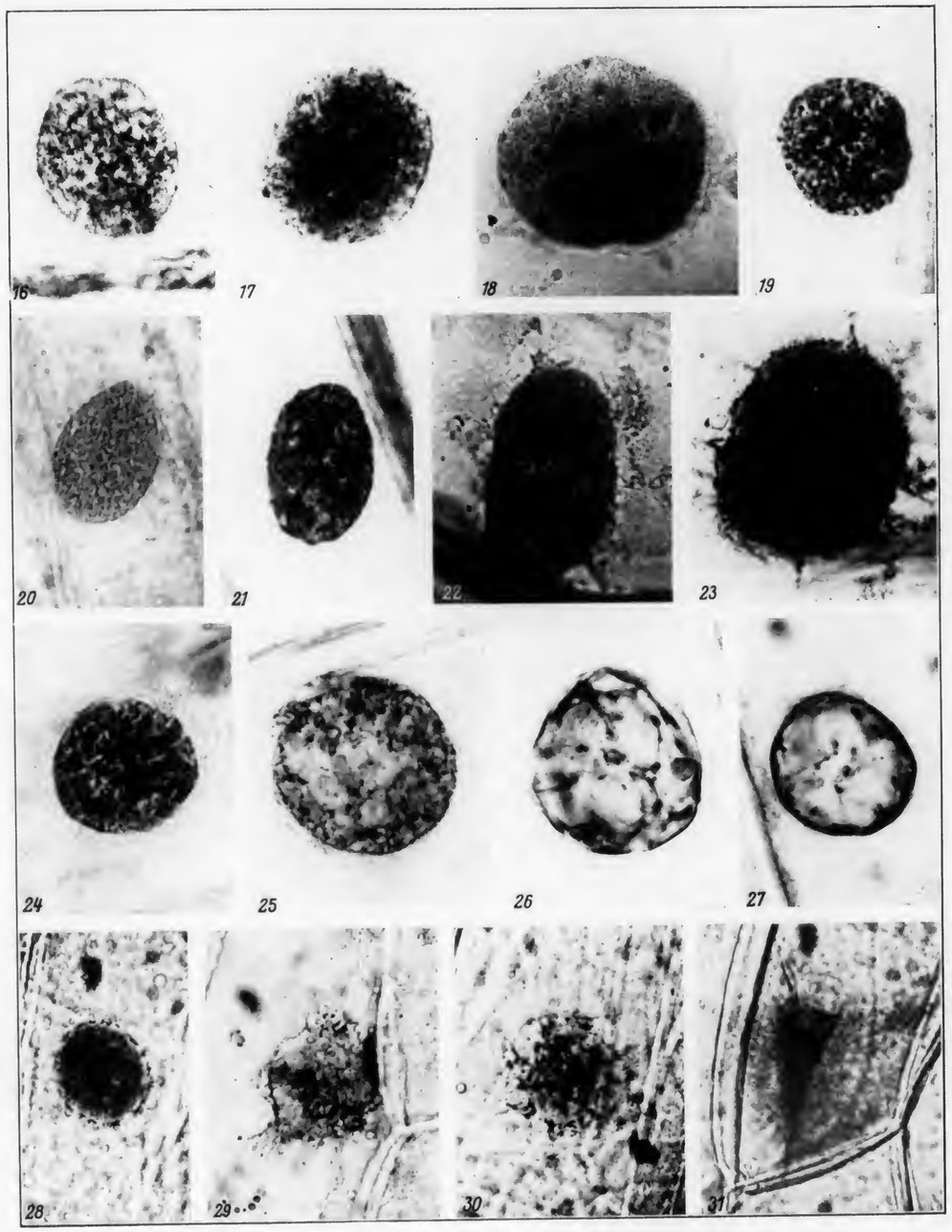

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INTENTIONAL SECOND EXPOSURE

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The Flora of Seven Mile Beach, New Jersey

Flora S. Fender

Part I: General Discussion

INTRODUCTION

Seven Mile Beach is one of the coastal islands off the shore of Cape May County, New Jersey. It lies parallel to the coast in a NE–SW direction, and has accumulated the greatest land-mass on the northern end. No detailed study of the plants of the island has previously been made, either as such or in any work dealing with the flora of the entire state. The present survey was therefore undertaken with a twofold aim: (1) to compile a comprehensive list of the plants of the island, and (2) to compare the flora of Seven Mile Beach with that of southern New Jersey as a whole, laying particular stress on the coastal islands of Cape May County.

EARLY BOTANICAL WORK

In the earliest botanical surveys of New Jersey, Willis's "Catalogue of the Plants Growing without Cultivation in New Jersey" (2nd ed., 1877), and Britton's catalogue of the same area (1881), no coast island is mentioned except Atlantic City. Stone's "Plants of Southern New Jersey" (1910) includes 149 species of native plants from the island, based on specimens which are at present, almost in their entirety, in the herbarium of the Philadelphia Botanical Club.

RECENT BOTANICAL WORK

Since Stone's publication (7), occasional collecting on the island by W. M. Benner, R. R. Dreisbach, J. M. Fogg, Jr., C. Knopf, B. Long, F. W. Pennell, H. E. Stone, and a few others brought the number of species and varieties of plants, both native and introduced, to 255. Further collecting from April, 1935 to September, 1937, has increased this figure to 367. Those reported by Otway Brown, who has botanized on the island since 1899, bring the total number to 415. According to Mr. Brown's statement, the best stations have been destroyed, but he has unfortunately not kept specimens from them.

In connection with the present study, the herbaria of the Philadelphia Botanical Club and the University of Pennsylvania were inspected for plants from Seven Mile Beach, and, for comparison, from the other coastal islands of New Jersey. In the former herbarium, 253 species were represented from Seven Mile Beach. All but two or three of the additional collection of 111 species have also been deposited there. The University herbarium contained only 23 specimens from the island, which number has grown to 505, representing 277 species. These

IRREGULAR PAGINATION

were obtained on collecting trips taken on an average of every two weeks during the growing season from early April, 1935, to June, 1936. Scattered trips have been taken since then. An attempt was made to cover all habitats at all seasons.

TOPOGRAPHY

There are three towns situated on Seven Mile Beach, Avalon and Peermont contiguous on the north end of the island, and Stone Harbor about a mile from its southern tip. Since the towns are not very large, much of the land even within their limits seems undisturbed. Between the two settlements are houses scattered along the connecting highway, but to the east is the very interesting region of the tall dunes. This region has apparently been little disturbed in the past four or five years, but was much cut when the now-abandoned railroad ran through. Directly below Stone Harbor is another patch of woods, but a great deal of the land behind the dunes to the southwest of the town is covered with brackish marsh.

HABITATS

Beach: Gently sloping beach edges the island on the side toward the sea and up the bay shore at each end. In common with other sand beaches, scattered plants grow above the reach of the storm waves. These are chiefly *Cakile edentula* and *Xanthium echinatum, Arenaria peploides* var. *robusta*, an occasional *Euphorbia polygonifolia*, and *Salsola Kali* and *Atriplex arenaria* wherever the beach is low.

Salt Marsh: The land side of the island is edged with extensive salt marshes, characterized by *Spartina patens* and *Distichlis spicata*, with frequent patches of *Salicornia ambigua, S. herbacea*, and *S. mucronata*. The edge of the marsh is fringed with thickets of *Baccharis halimifolia* and *Iva frutescens* var. *oraria*, and near them grow less frequent but just as typical marsh plants, notably *Limonium carolinianum, Pluchea camphorata*, and *Aster tenuifolius*. *Spergularia leiosperma* and *Suaeda linearis* grow in more sandy places.

Salt Marsh Islands: The wide marshes have many scattered islands. Two of these, Crab Island and an unnamed one to the north of it, have been explored. They are much like the well developed thickets of the mainland, consisting mainly of *Quercus stellata* and *Juniperus virginiana*. The latter is in such abundance that the islands are locally called " cedar ridges ".

Sand Dunes: The sand dunes are of two types, grass-bound and tree-bound. The former are those nearest the sea, and none has been observed more than 25 feet high, although the average is about 15 feet. The sand-binder is marram or beach grass (*Ammophila breviligulata*), but other characteristic dune species are: *Triplasis purpurea, Spartina patens, Panicum amarum, Euphorbia polygonifolia, Oenothera biennis*, and *Solidago sempervirens*. The dunes farther from the sea are most frequently formed around a clump of *Prunus serotina*. After the dune has been started by the cherries, there is sufficient protection for *Sassafras variifolium, Rhus Toxicodendron* (the bush form), *Vitis aestivalis, V. labrusca*, and *Psedera quinquefolia* to become established. These, in turn, prevent the west wind, which is the prevailing one on this coast, from blowing back the sand. As a result, tree-bound dunes become as high as 45 feet. They are usually bound by *Ammophila* on the side facing the sea, except when they occur within the woods, where they are rarely very tall.

Sand Dune Hollows: These are of three types, open sand, grass-filled, and thicket-filled. In the first grow scattered plants of *Panicum Lindheimeri* var. *fasciculatum, Cenchrus pauciflorus, C. tribuloides, Juncus bufonius, Strophostyles helvola, S. umbellata, Euphorbia polygonifolia*, and *Polygonella articulata*. The second are thickly covered with *Ammophila breviligulata*, with scattered *Myrica carolinensis* and *Vitis aestivalis*. The bayberry forms the thickets of the third type. In them grow *Achillea Millefolium, Erechtites hieracifolia, Solanum nigrum* and *Phytolacca americana*. The central bushes of these thickets often die off from some undetermined cause, and in the protected spot thus formed grow *Achillea Millefolium, Erechtites hieracifolia, Aster lateriflorus*, and *Oenothera fruticosa*. They are the forerunners of the woods formation into which such a thicket ultimately grows. Of course, every intermediate form exists.

Woods: Woods have developed behind the tall dunes. In fact, woods and dunes are interdependent, each affording the necessary protection for the other. Harshberger (4) observes that the dune finally destroys the woods, and it is true that one can observe a great deal of buried forest. However, the advance cannot be as swift as he implies, since then there would be a succession of very short-lived woods, and the present one, by tree-ring count, must be over 200 years old. The dominant trees are *Juniperus virginiana* var. *crebra, Quercus stellata* and *Q. falcata, Sassafras variifolium, Amelanchier oblongifolia, Prunus serotina*, and *Rhus copallina*. *Rhus Toxicodendron* is the most frequent underbush, and *Smilax rotundifolia* the most constant vine, sometimes making the woods absolutely impassable. The soil beneath these trees is often dry sand, when the woods are somewhat open, but just as often it is a rich, dark leaf-mold, made light in texture by the unusual percentage of sand.

Low places in the woods are most likely to support *Myrica carolinensis, Nyssa sylvatica, Vaccinium atrococcum, V. corymbosum*, and *Diospyros virginiana*. One large and well established swamp is dominated by *Acer rubrum* var. *tridens* and contains a clump of *Liquidambar Styraciflua*.

Fresh Marshes: These occur at intervals along the edge of the woods and in various other dune-protected spots. They usually contain *Typha latifolia, T. angustifolia, Hibiscus Moscheutos, Sium suave*, and, occasionally, *Scirpus Eriophorum* or *Iris versicolor*. The more extensive ones are often merely wet, and are covered with a mixture of *Spartina patens* and *Panicum virgatum*.

Elements in the Flora—New Jersey

Undifferentiated Coastal Plain

The most prominent element in the flora of Seven Mile Beach is that of the region of southern New Jersey which is called here, for want of a better name, the Undifferentiated Coastal Plain. This name is used to distinguish it from specialized pine barren and maritime districts and to link it with the similar widespread formation of the Atlantic Coast of North America from New Jersey to Florida. This region includes the Middle District, Cape May District and Coast Strip of Dr. Stone (7). The combination of these undeniably similar districts has been made because the basis of Stone's division was the Piedmont element rather than the characteristically coastal plain plants, whereas the other two districts (Pine Barren and Maritime) are on such a "native" basis. Moreover, the comparative absence of Piedmont species from the Coast Strip and abundance of them in the Middle District seems rather a question of area available than of ability to support them. More than two-fifths of the plants on Seven Mile Beach belong to the region of the Undifferentiated Coastal Plain, a proportion which would be expected from the situation of the island. Of this element may be noted: *Thelypteris palustris, Juniperus virginiana* var. *crebra, Typha latifolia, T. angustifolia, Elymus virginicus, Panicum clandestinum, P. virgatum, Cyperus Grayi, Carex Swanii, C. comosa, Juncus effusus* var. *solutus, Iris versicolor, Salix nigra* var. *falcata, Quercus phellos, Rumex verticillatus, Ranunculus sceleratus, Liquidambar Styraciflua, Rubus argutus, Rosa virginiana, Prunus serotina, Strophostyles helvola, S. umbellata, Oxalis stricta, Rhus copallina, R. Toxicodendron, Euonymus americanus, Hibiscus Moscheutos, Oenothera fruticosa, Diospyros virginiana, Convolvulus sepium, Salvia lyrata, Lycopus americanus, Tecoma radicans, Galium pilosum, G. Claytoni, Mitchella repens, Sambucus canadensis, Mikania scandens, Aster lateriflorus, Helianthus giganteus,* and *Bidens connata.* Because of their great interest, the Piedmont plants have been separated out as a subdivision of the Undifferentiated Coastal Plain. Thirteen of these have been discovered on the island: *Poa cuspidata, Smilacina stellata, Morus rubra, Silene stellata, Aquilegia canadensis, Phaseolus polystachyus, Celastrus scandens, Geranium Robertianum, Verbena urticaefolia, Teucrium canadense* var. *virginicum, Scrophularia lanceolata, Galium circaezans,* and *G. triflorum.* This is a small list as compared with the 265 species in West Jersey, but they are included in an area 1.5 miles long and 500 feet wide. Furthermore, it is interesting that some of these are very well established. Particularly is this true of *Poa cuspidata, Aquilegia canadensis,* and *Celastrus scandens,* which range almost the length of the woods.

General

About one third of the plants of the island are of widespread occurrence in southern New Jersey. Among these have been included species which are not strictly universal, i. e., those which commonly enter the western and southern part of the Pine Barrens, not its eastern, more thoroughly "barren" areas. Some of the most common general species on the island are as follows: *Triplasis purpurea, Cyperus cylindricus, Carex Longii, Scirpus americanus, S. Eriophorum, Juncus dichotomus, J. canadensis, Smilax rotundifolia, Myrica carolinensis, Quercus stellata, Q. falcata, Polygonella articulata, Magnolia virginiana, Sassafras variifolium, Lepidium virginicum, Pyrus arbutifolia, Amelanchier oblongifolia, Rubus cuneifolius, Prunus maritima, Apios tuberosa, Linum medium, Acer rubrum* var. *tridens, Helianthemum canadense, Lechea Leggettii, Ludvigia alternifolia, Nyssa sylvatica, Vaccinium corymbosum, V. atrococcum, Linaria canadensis, Eupatorium album, E. hyssopifolium, E. rotundifolium* var. *ovatum, Solidago suaveolens, S. nemoralis, S. tenuifolia, Aster novi-belgii, Erigeron ramosus, Gnaphalium obtusifolium,* and *Krigia virginica.*

Maritime

Nearly one-fifth of the native plants in number of species, and by far the greatest number of individuals, are maritime. A characteristic list includes: *Ammophila breviligulata, Andropogon scoparius* var. *polycladus, Cenchrus tribuloides, Distichlis spicata, Elymus virginicus* var. *halophilus, Festuca rubra, Panicum amarum, Spartina alterniflora* var. *pilosa, S. patens, Cyperus filicinus, Juncus Gerardi, Atriplex arenaria, Salicornia ambigua, S. herbacea, S. mucronata, Salsola Kali, Suaeda linearis, Arenaria peploides* var. *robusta, Spergularia leiosperma, Cakile edentula, Euphorbia polygonifolia, Limonium carolinianum, Sabatia stellaris, Plantago oliganthos, Baccharis halimifolia, Iva frutescens* var. *oraria, Pluchea camphorata, Solidago graminifolia, S. sempervirens, Aster tenuifolius,* and *Xanthium echinatum.*

Elements in the Flora—Introduced

Fifty introduced species have been collected from the island. The most prominent of these are the yarrow (*Achillea Millefolium*) in the thickets and the Japanese honeysuckle (*Lonicera japonica*) in the woods. Most of the others are in local patches near house-sites or on filled-in ground.

Elements in the Flora—North America

By far the greater part of the plants on the island represent species which are widespread in eastern North America, that is, they range from Labrador, Nova Scotia, or Maine to Virginia or Florida. About one quarter (26%) belong to the southern or coastal plain element, which reaches its northern limits in New Jersey, Long Island, and southeastern Massachusetts. Only seven per cent of the species are northern, that is, reaching their southern limits in New Jersey or the adjacent states. One per cent belongs to a restricted element which grows only in New Jersey and adjoining states. Except for a slightly greater emphasis on the widespread plants, these percentages correspond in general to those obtained from Stone's (7) figures for all of southern New Jersey:

	Seven Mile Beach	Southern New Jersey
Widespread	64%	54%
Southern	26%	35%
Northern	7%	9%
Restricted	3%	2%

It is to be noted that the widespread and southern elements include certain Piedmont species which have traveled down the mountains to Georgia, and which therefore have a northern affinity despite their range, and a corresponding significance when growing on the coastal plain in southern New Jersey. These include: *Poa cuspidata, Morus rubra, Silene stellata, Aquilegia canadensis, Phaseolus polystachyus, Celastrus scandens, Verbena utricaefolia, Teucrium canadense* var. *virginicum, Galium circaezans,* and *G. triflorum.*

Summary

The results of the study are summarized in the following table:

Total number of species and varieties known from Seven Mile Beach		416
Introduced	63	
Native	353	
Species added by this study to Seven Mile Beach		111
Introduced	37	
Native	74	
Species added by this study to the coast island flora of N. J.		34
Introduced	17	
Native	17	
New Jersey elements in the native flora		
General	128	36.2%
Undifferentiated Coastal Plain	162	45.8%
Pine Barren	1	0.2%
Maritime	61	17.8%

ACKNOWLEDGMENTS

The writer's deepest gratitude is due to Dr. John M. Fogg, Jr. for the sympathetic guidance which has made this paper possible. She also wishes to express appreciation for the invaluable suggestions of Mr. Bayard Long.

Part II: Annotated List of the Vascular Flora

EXPLANATION OF THE TABLES

Range: The range in North America is self-explanatory. North and south, rather than westward extension has been considered. The terms employed in the New Jersey ranges have been discussed under *Elements in the Flora.* No

attempt has been made to give the New Jersey range of introduced species because the collections are very inadequate.

Habitat: The habitats are those in which specimens were collected, except when the plant was not personally observed or there was no habitat on the label of the collector. In that case, the plant's usual habitat was combined with those available on the island to make the probable one.

Localities and Collectors on Seven Mile Beach: Directions have been omitted in these in order to save space. A combination of " woods " and " Peermont " usually means south of Peermont. The sand dunes and marshes are only rarely within the towns. On the other hand, the swamp and woods in Avalon are surrounded by houses. The initials of the collector follow in parentheses.

Nomenclature and Synonymy: The International Rules of Botanical Nomenclature have been followed. Synonyms are included in parentheses when the name used differs from that given in the last edition of Gray's Manual (1908).

Symbols Employed in the Tables

Range—North America (= NA)

N = northern (southern limit in N.J., Del., Md., or Va.)
S = southern (northern limit in N.J., L.I., Conn. or Mass.)
W = widespread along the Atlantic seaboard of N.A.
R = restricted to N.J. and adjoining States

Range—Southern New Jersey (= NJ)

PB = Pine Barren
CP = Undifferentiated Coastal Plain
Ma = Maritime
G = General in S. N. J.
In = Introduced

Habitat

Beach = beach
Dr Op Sd = dry open sand
Fr Mar = fresh marsh
Sd Dune = sand dune
Sd Du Ho = sand dune hollow
Sal Mar = salt marsh
Sal Mar Bord = salt marsh border
Swamp = swamp
Thick = thicket
Wood = woods
Wet Sd = Wet sand
Open Wood = dry, sandy, opening in woods

Localities on Seven Mile Beach

Ava = Avalon
Peer = Peermont
St. Har = Stone Harbor
Cr. I. = Crab Island
n Cr. I. = island north of Crab Is.

Collectors and Herbaria

WB = Walter Benner (University of Pennsylvania, Philadelphia Botanical Club)
JBB = J. B. Brinton (U of P)
SB = Stewardson Brown (PBC)
RRD = R. R. Dreisbach (PBC)
FF = Flora Fender (U of P, PBC, Morris Arboretum)

JMF = John M. Fogg, Jr. (U of P, PBC)
AJ = Albrecht Jahn (PBC)
IK = Ida Keller (PBC)
CK = Charles Knopf (U of P)
BL = Bayard Long (PBC, U of P)
FWP = F. W. Pennell (PBC, U of P)
HS = Hugh Stone (PBC)
WS = Witmer Stone (PBC)
SSVP = S. S. Van Pelt (PBC)
CSW = C. S. Williamson (PBC)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
POLYPODIACEAE				
Asplenium platyneuron (L.) Oakes	W	G	Woods	Peer (WS, CSW)
Thelypteris palustris Schott (*Aspidium Thelypteris*)	W	CP	Fr Mar	Peer (FF, FWP, WS)
Pteridium aquilinum (L.) Kuhn (*Pteris aquilina*)	W	G	Thick	Peer (FF)
Onoclea sensibilis L.	W	CP	Swamp	St Har (FF)
OSMUNDACEAE				
Osmunda regalis L.	W	G	Swamp	St Har (FF)
O. cinnamomea L.	W	G	Swamp	Ava (FF)
OPHIOGLOSSACEAE				
Botrychium dissectum Spreng. f. obliquum (Muhl.) Fernald (*B. obliquum* var. *dissectum*)	S	G	Woods	Peer (FF, CK)
EQUISETACEAE				
Equisetum arvense L.	W	CP	Dr Op Sd	Ava (FF)
LYCOPODIACEAE				
Lycopodium inundatum L. var. Bigelovii Tuckerm.	R	G	Sd Du Ho	Peer (FWP)
PINACEAE				
Pinus rigida Mill.	W	G	Dr Op Sd	Peer (FF)
Juniperus virginiana L. var. crebra Fernald	N	CP	Woods Thick	Peer (FF, WS), St. Har (FF), Cr I (FF, JMF)
TYPHACEAE				
Typha latifolia L.	W	CP	Fr Mar	Ava (FF, JMF, SB), Peer (FWP), St Har (WB)
T. angustifolia L.	W	CP	Fr Mar	Ava (SSVP), Peer (FF, JMF, SSVP)
NAIADACAE				
Ruppia maritima L.	W	Ma	Sal Mar Pools	Peer (WS), St Har (SSVP, CSW)
Zostera marina L.	W	Ma	Sal Mar	reported by O. Brown
GRAMINEAE				
Poa annua L.	W	In	Dr Op Sd	Ava (BL)
P. pratensis L.	W	In	Open Woods	Peer (FF)
P. cuspidata Nutt. (*P. brachyphylla*)	S	CP	Woods	Peer (FF)
P. compressa L.	W	In	Woods	Peer (FF)
Eragrostis caroliniana (Spreng.) Scribn. (*E. Purshii*)	W	G	Sd Dune	Ava (FF, JMF, HS)
E. pectinacea (Michx.) Steud. var. spectabilis Gray	W	Ma	Sd Dune	Ava (JMF), Peer (FWP), St Har (FF)
Distichlis spicata (L.) Greene	W	Ma	Sal Mar	Ava (FF, HS), Peer (SSVP), St Har (FF, RRD)
Uniola laxa (L.) BSP.	S	CP	Wet Sd	Peer (FWP)
Phragmites communis Trin.	W	CP	Wet Sd	St Har (FF)
Triodia flava (L.) Smyth (*Tridens flavus*)	S	CP	Woods	Ava (FF)
Bromus tectorum L.	W	In	Sd Dune, Dr Op Sd	Ava (Clarke), Peer (FF)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Festuca octoflora Walt.	W	G	Dr Op Sd	Peer (FF)
F. myuros L.	W	In	Dr Op Sd	Peer (FF)
F. rubra L.	W	Ma	Dr Op Sd, Thick	Ava (BL), Peer (FF), CrI (FF), St Har (FF, SSVP)
F. elatior L.	W	In	Dr Op Sd	reported by O. Brown
Glyceria septentrionalis Hitchc.	W	CP	Fr Mar	Peer (WS), St Har (SSVP)
G. obtusa (Muhl.) Trin.	N	G	Swamp	Peer (HS)
G. pallida (Torr.) Trin.	W	CP	Swamp	Peer (FF)
Triplasis purpurea (Walt.) Chapm.	W	G	Sd Dune, Dr Op Sd	Ava (FF, JMF, HS), Peer (SSVP), St Har (FF)
Agropyron repens (L.) Beauv.	W	In	Dr Op Sd	St Har (WB)
Elymus australis S & B var. glabriflorus Wiegand (*E. canadensis*, in part)	W	Ma	Sal Mar Bord	Ava (JMF, HS), St Har (WB)
E. virginicus L.	W	CP	Dr Op Sd, Thick	Ava (FF), Peer (FWP), Cr I (FF, JMF)
E. v. var. halophilus (Bickn.) Wiegand	N	Ma	Dr Op Sd, Thick	Peer (FF, FWP), Cr I (FF)
Sphenopholis obtusata (Michx.) Scribn.	S	CP	Dr Op Sd	Peer (FF, HS), St Har (SSVP)
Aira praecox L.	R	In	Dr Op Sd	Ava (BL)
Holcus lanatus L.	W	In	Dr Op Sd	Ava (SSVP), Peer (FF, HS)
Danthonia spicata (L.) Beauv.	W	G	Open Woods	Peer (FF, WS)
Ammophila breviligulata Fernald (*A. arenaria*)	W	Ma	Sd Dune	Ava (JMF, Kaji), Peer (FF), St Har (FF, HS)
Agrostis stolonifera L. (*A. alba* var. *vulgaris*)	N	In	Dr Op Sd	St Har (WB)
A. perennans (Walt.) Tuckerm.	W	CP	Woods	Ava (FF)
Cinna arundinacea L.	W	CP	Woods	Peer (WS)
Aristida purpurascens Poir.	S	G	Open Woods	Peer (SSVP)
Cynodon Dactylon (L.) Pers.	S	In	Dr Op Sd	Ava (BL)
Spartina cynosuroides (L.) Roth	S	Ma	Sal Mar	Ava (HS)
S. alterniflora Loisel var. pilosa Merr. (*S. glabra* var. *pilosa*)	W	Ma	Sal Mar	Ava (JMF), Peer (FF), St Har (RRD, FF)
S. patens (Ait.) Muhl.	W	Ma	Sal Mar, Sd Dune	Ava (FF, HS), Peer (FF), St Har (FF, SB, Jahn)
S. p. var. juncea (Michx.) Hitchc.	W	Ma	Sal Mar, Sd Dune	Ava (JBB, JMF, Witte), St Har (FF)
Anthoxanthum odoratum L.	W	In	Dr Op Sd	Ava (FF), Peer (CK)
Leersia oryzoides (L.) Sw.	W	CP	Swamp	Peer (WS)
Digitaria sanguinalis (L.) Scop.	W	In	Dr Op Sd	Ava (FF, JMF), St Har (WS)
Paspalum setaceum Michx.	W	G	Dr Op Sd	Peer (FWP)
P. pubescens Muhl.	S	G	Dr Op Sd	St Har (WB)
Panicum verrucosum Muhl.	S	G	Wet Sd	reported by O. Brown
P. microcarpon Muhl.	S	CP	Woods	Ava (SSVP), Peer (WS)
P. Lindheimeri Nash. var. fasciculatum (Torr.) Fernald (*P. huachucae*, *P. tennesseense*)	W	CP	Woods, Sd Dune	Ava (SSVP), Peer (FF, SSVP)
P. meridionale Ashe	S	G	Woods	Peer (HS)
P. lanuginosum Ell.	S	CP	Sal Mar Bord, Woods	Ava (HS), Peer (SSVP, WS)
P. villosissimum Nash	S	G	Woods	Ava (SSVP)
P. Addisonii Nash	S	G	Dr Op Sd	Peer (WS)
P. sphaerocarpon Ell.	W	G	Dr Op Sd	Ava (SSVP), Peer (SSVP)
P. scoparium Lam.	S	G	Woods, Thick	Ava (FF, HS), Peer (SB, FF), St Har (WB, Jahn)
P. clandestinum L.	W	CP	Thick	Peer (FF, SSVP)
P. dichotomiflorum Michx.	W	CP	Wet Sd	Peer (FWP)
P. amarum Ell.	S	Ma	Sd Dune	Ava (FF, JMF, HS), St Har (FF)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Panicum amarulum H. & C.	S	Ma	Dr Op Sd	St Har (FF)
P. virgatum L.	W	CP	Dry or Wet Sd	Ava (JMF), Peer (FF), St Har (WS)
P. v. var. spissum Linder	N	G	Dr Op Sd	Ava (FF)
P. longifolium Torr.	W	G	Wet Sd	reported by O. Brown
P. condensum Nash	S	CP	Fr Mar	Peer (WS)
Echinochloa Walteri (Pursh) Nash	S	Ma	Sal Mar Bord	Ava (FF, CK)
Setaria geniculata (Lam.) Beauv. (*S. imberbis* var. *perennis*)	S	Ma	Sal Mar Bord	Peer (FWP)
S. viridis (L.) Beauv.	W	In	Dr Op Sd	Ava (FF)
Cenchrus pauciflorus Benth. (*C. carolinianus*)	W	G	Sd Dune, Dr Op Sd	Ava (FF), Peer (FF)
C. tribuloides L.	S	Ma	Sd Dune	Ava (FF, JMF, BL), St Har (RRD)
Andropogon scoparius Michx. var. polycladus S. & B. (*A. s.* var. *littoralis*)	S	Ma	Dr Op Sd	Ava (FF), Peer (FWP), St Har (FF)
A. glomeratus (Walt.) BSP.	S	G	Dr Op Sd	reported by O. Brown
A. virginicus L.	S	G	Dr Op Sd	reported by O. Brown
CYPERACEAE				
Cyperus filicinus Vahl (*C. Nuttallii*)	W	Ma	Sal Mar, Fr Mar	Ava (FF, JMF, HS), Peer (FWP, SSVP)
C. ferax Rich.	S	Ma	Sd Dune, Dr Op Sd	Ava (FF, JMF), Peer (FWP, SSVP), St Har (FF, SSVP)
C. esculentus L.	W	CP	Sd Du Ho	Ava (FF, JMF, HS), Peer SSVP)
C. strigosus L.	W	CP	Fr Mar Wet Sd	Peer (FWP), St Har (FF)
C. cylindricus (Ell.) Britton	S	G	Dr Op Sd	Peer (FF, SSVP), n Cr I (FF)
C. ovularis (Michx.) Torr.	S	CP	Dr Op Sd	reported by O. Brown
C. Grayi Torr.	R	CP	Sd Dune, Dr Op Sd	Peer (FF, SSVP), St Har (SB, FF, SSVP)
C. filiculmis Vahl. var. macilentus Fernald	W	G	Dr Op Sd	Peer (FF), St Har (WB)
Eleocharis parvula (R. & S.) Link (*Scirpus nanus*)	W	Ma	Sal Mar	Ava (FWP)
E. Smallii Britton (*E. palustris,* in part)	N	CP	Fr Mar	Peer (reported in (7), but no specimen seen)
E. halophila Fernald and Bracken (*E. palustris,* in part)	N	Ma	Fr Mar	Peer (FF), St Har (FF)
E. calva Torr. (*E. palustris* var. *calva*)	W	CP	Fr Mar,	Peer (SSVP)
Stenophyllus capillaris (L.) Britton	W	CP	Dr Op Sd	reported by O. Brown
Fimbristylis castanea (Michx.) Vahl.	S	Ma	Wet Sd	Ava (HS), Peer (FWP, SSVP), St Har (SB, SSVP)
F. mucronulata (Michx.) Blake (*F. autumnalis*)	S	G	Fr Mar	reported by O. Brown
Scirpus americanus Pers.	W	G	Fr Mar, Wet Sd	Ava (RRD, JMF), Peer (FF, WS), St Har (WB, HS, (SSVP)
S. validus Vahl	W	CP	Fr Mar	Peer (SSVP)
S. robustus Pursh	S	Ma	Sal Mar	St Har (SSVP)
S. Eriophorum Michx.	S	G	Fr Mar	Peer (FF, FWP), Ava (FF), St Har (FF)
Rynchospora capitellata (Michx.) Vahl (*R. glomerata,* in part)	W	G	Wet Sd	Peer (FWP)
Mariscus mariscoides (Muhl.) Kuntze (*Cladium mariscoides*)	W	G	Fr Mar	reported by O. Brown

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Carex scoparia Schkuhr	W	G	Wet Sd	St Har (CSW)
C. Longii Mackenzie (*C. albolutescens*)	W	G	Thick, Dr Op Sd	Ava (JBB), Peer (FF, WS), St Har (JBB)
C. hormathodes Fernald	N	Ma	Sal Mar	Ava (FF, SSVP), Peer (SSVP)
C. silicea Olney	N	Ma	Sd Du Ho	Peer (WS), St Har (Jahn, SSVP)
C. alata Torr.	W	CP	Sal Mar Bord	Ava (SSVP), Peer (FF, WS)
C. vulpinoidea Michx.	W	CP	Fr Mar	Ava (SSVP), Peer (WS), St Har (FF)
C. crinita Lam.	W	CP	Fr Mar	reported by O. Brown
C. Swanii (Fernald) Mackenzie (*C. virescens* var. *Swanii*)	W	CP	Fr Mar	Ava (JBB, SSVP), Peer (FF, WS)
C. nigro-marginata Schwein.	S	CP	Woods	Peer (FF)
C. pennsylvanica Lam.	W	G	Woods	reported by O. Brown
C. lanuginosa Michx.	S	CP	Sal Mar Bord	Peer (FF, SSVP)
C. comosa Boott	W	CP	Swamp	Ava (JBB), Peer (FF, SSVP)
C. lurida Wahlenb.	W	G	Swamp	Peer (SSVP)
JUNCACEAE				
Juncus bufonius L.	W	CP	Sd Du Ho, Dr Op Sd	Ava (RRD, BL), Peer (FF, WS), St Har (CSW, SSVP)
J. Gerardi Loisel.	W	Ma	Sal Mar, Sd Du Ho	Peer (FF, SSVP), St Har (WB)
J. tenuis Willd.	W	CP	Open Woods	Peer (FF)
J. dichotomus Ell.	S	G	Fr Mar	Ava (RRD), Peer (FF, FWP), St Har (JBB)
J. effusus L. var. solutus Fernald & Wiegand	W	G	Fr Mar	Peer (FF)
J. canadensis J. Gay	W	G	Fr Mar, Wet Sd	Peer (FF, FWP)
J. scirpoides Lam.	S	CP	Fr Mar	Ava (RRD), Peer (FF, WS) St Har (WB, Jahn)
J. acuminatus Michx.	W	G	Fr Mar	Ava (SSVP), Peer (FWP, WS)
J. aristulatus Michx.	S	G	Fr Mar	Peer (FWP)
Luzula campestris (L.) DC. var. bulbosa A. Wood	S	CP	Wet Sd	Peer (FF)
LILIACEAE				
Hemerocallis fulva L.	W	In	Dr Op Sd	St Har (FF)
Lilium superbum L.	W	G	Fr Mar	Peer (FF, FWP)
Asparagus officinalis L.	W	In	Woods	Peer (FF)
Smilacina stellata (L.) Desf.	N	CP	Open Wood	Peer (FF, SB)
Maianthemum canadense Desf.	W	CP	Woods	Peer (FF)
Polygonatum biflorum (Walt.) Ell. (*P. commutatum*)	W	CP	Woods	Ava (FF, FWP), Peer (FF, WS)
Aletris farinosa L.	W	G	Woods	St Har (SB)
Smilax rotundifolia L.	W	G	Woods	Peer (FF)
S. glauca L.	S	G	Thick	Peer (FF)
IRIDACEAE				
Iris versicolor L.	W	CP	Fr Mar	Peer (FF, WS, SSVP), Ava (FF)
Sisyrinchium gramineum Curtis	W	CP	Fr Mar	Ava (FF), Peer (FF, SSVP)
S. atlanticum Bicknell	W	G	Wet Sd	Ava (SSVP), Peer (FF)
ORCHIDACEAE				
Spiranthes vernalis Engelm & Gray	S	CP	Wet Sd	Ava (IK, FWP), Peer (FF)
S. cernua (L.) Richards.	W	G	Wet Sd	Peer (FWP)
SALICACEAE				
Salix nigra Marsh. var. falcata (Pursh.) Torr.	N	CP	Swamp, Sd Du Ho	Peer (FF, WS), Ava (FF)
Populus alba L.	W	In	Dr Op Sd	St Har (FF)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
MYRICACEAE				
Myrica carolinensis Mill.	W	G	Sd Du Ho, Woods	Peer (FWP), St Har (FF), Cr I (JMF) Ava (FF)
M. asplenifolia L.	W	G	Dr Op Sd	Ava (BL, HS), St Har (FF)
FAGACEAE				
Quercus stellata Wang.	S	G	Woods	Ava (SSVP), Peer (SB, FF), n Cr I (JMF)
Q. falcata Michx.	S	G	Woods	Peer (FF)
Q. phellos L.	S	CP	Woods	Peer (FF)
URTICACEAE				
Celtis occidentalis L.	W	CP	Woods	Peer (FF, SSVP), Ava (FF)
Morus rubra L.	W	CP	Woods	Peer (FF, WS)
Boehmeria cylindrica (L.) Sw.	W	CP	Woods	Peer (FWP)
POLYGONACEAE				
Rumex verticillatus L.	W	CP	Swamp	Ava (FWP), Peer (FF, SSVP)
R. crispus L.	W	In	Dr Op Sd	Peer (FF)
R. Acetosella L.	W	In	Woods, Dr Op Sd	Ava (FF, Kaji), Peer (FF), St Har (FF)
Polygonum prolificum (Small) Robinson	W	Ma	Sal Mar Bord	Peer (FF, WS), St Har (SSVP)
P. aviculare L.	W	G	Sal Mar, Fr Mar	Ava (FF, HS), St Har (FF)
P. pennsylvanicum L.	S	G	Wet Sd	Ava (Kaji)
P. punctatum Ell. (*P. acre*)	S	CP	Fr Mar	Ava (FF, JMF), Peer (FF, WS) St Har (FF)
P. Persicaria L.	W	In	Sal Mar Bord	Ava (RRD, JMF)
P. sagittatum L.	W	CP	Fr Mar	Peer (FWP)
Polygonella articulata (L.) Meisn.	W	G	Sd Dune, Wet Sd	Peer (FF), St Har (FF)
CHENOPODIACEAE				
Cycloloma atriplicifolium (Spreng) Coult.	W	In	Beach	Ava (FF, JMF), St Har (SB, FF)
Chenopodium ambrosioides L.	W	In	Beach	Peer (FWP), Cr I (FF, JMF)
C. album L.	W	In	Sd Du Ho	Ava (FF, BL)
C. Boscianum Moq.	W	CP	Woods	reported by O. Brown
C. leptophyllum Nutt.	N	CP	Open Wood	Ava (FF, BL)
Atriplex patula L. var. hastata (L.) Gray	W	CP	Sal Mar Bord	Ava (FF), Peer (FF), St Har (FF), n Cr I (FF, JMF)
A. arenaria Nutt.	S	Ma	Beach	Ava (BL), Peer (FWP, WS), St Har (FF, SSVP), Cr I (FF, JMF)
Bassia hirsuta (L.) Aschers.	R	Ma	Sal Mar	Ava (FWP)
Salicornia mucronata Bigel.	W	Ma	Sal Mar	Ava (FF), Peer (FF, FWP), St Har (SSVP)
S. herbacea L. (*S. europaea*)	W	Ma	Sal Mar	Ava (FF, JMF, Kaji), Peer (FWP), n Cr I (FF, JMF)
S. ambigua Michx.	W	Ma	Sal Mar	Peer (SSVP), St Har (FF), n Cr I (FF, JMF)
Suaeda linearis (Ell.) Moq.	W	Ma	Sal Mar	Peer (FWP), St Har (FF), n Cr I (FF, JMF)
Salsola Kali L.	W	Ma	Beach	Ava (FF, JMF, RRD), Peer (FWP), St Har (FF)
PHYTOLACCACEAE				
Phytolacca americana L.	W	CP	Thick	Peer (FF), St Har (WB)
CORRIGIOLACEAE				
Scleranthus annuus L.	W	In	Dr Op Sd	Ava (RRD)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
AIZOACEAE				
Sesuvium maritimum (Walt.) BSP.	S	Ma	Sal Mar	Ava (FWP), St Har (FF)
CARYOPHYLLACEAE				
Spergularia leiosperma (Kindberg) F. Schmidt (*S. marina*, in part)	N	Ma	Sal Mar	Peer (FF), St Har (WB)
Sagina decumbens (Ell.) T. & G.	N	CP	Sd Du Ho	Ava (CSW), St Har (CSW)
Arenaria peploides L. var. robusta Fernald	N	Ma	Beach	Ava (FWP), Peer (FF, WS), St Har (SSVP & CSW)
Stellaria media (L.) Cyrill	W	In	Dr Op Sd	St Har (FF)
Cerastium vulgatum L.	W	In	Dr Op Sd	Peer (FF), St Har (FF)
C. semidecandrum L.	R	In	Dr Op Sd	Peer (CSW)
Silene pennsylvanica Michx.	S	CP	Woods	Peer (FF, CK, CSW), St Har (McCadden)
S. stellata (L.) Ait. f.	S	CP	Woods	Peer (FF)
Dianthus armeria L.	R	In	Dr Op Sd	St Har (FF, WB)
RANUNCULACEAE				
Ranunculus sceleratus L.	W	CP	Fr Mar	Peer (WS), Ava (FF)
Aquilegia canadensis L.	W	CP	Woods	Peer (FF, WS), St Har (CSW)
MAGNOLIACEAE				
Magnolia virginiana L.	S	G	Swamp	Peer (FF, WS & SSVP)
LAURACEAE				
Sassafras variifolium (Salisb.) Ktze.	W	G	Woods, Sd Dune	Peer (FF, WS), St Har (FF)
CRUCIFERAE				
Draba verna L.	S	In	Dr Op Sd	St Har (FF)
Lepidium virginicum L.	W	G	Dr Op Sd, Thick	Ava (FF, JMF), Peer (FF), St Har (FF), n Cr I (FF, JMF)
Capsella Bursa-pastoris (L.) Medic.	W	In	Open Woods	Peer (FF)
Cakile edentula (Bigel.) Hook.	W	Ma	Beach	Ava (RRD, JMF, Witte), Peer (FF), St Har (WB, SB)
Raphanus Raphanistrum L.	N	In	Dr Op Sd	Ava (FF)
Sisymbrium Thalianum (L.) J. Gay	S	In	Dr Op Sd	Peer (CK)
Cardamine parviflora L.	W	CP	Woods	Peer (CSW)
Arabis lyrata L.	W	CP	Woods	Ava (SB), Peer (WS)
CRASSULACEAE				
Sedum acre L.	W	In	Dr Op Sd	Ava (Kaji, BL)
HAMAMELIDACEAE				
Liquidambar Styraciflua L.	S	CP	Swamp	Peer (FF)
ROSACEAE				
Pyrus arbutifolia (L.) L. f.	S	G	Woods	Peer (FF)
P. a. var atropurpurea (Britton) Robinson	W	CP	Swamp	Ava (FF)
Amelanchier oblongifolia (T. & G.) Roem.	W	G	Woods	Peer (FF, WS), Ava (FF)
Fragaria virginiana Duchesne	W	CP	Woods	Ava (FF), Peer (FF, WS)
Potentilla recta L.	N	In	Dr Op Sd	Peer (FF)
P. pumila Poir.	W	CP	Dr Op Sd	reported by O. Brown
P. canadensis L.	W	CP	Dr Op Sd	reported by O. Brown
Geum canadense Jacq.	W	CP	Woods, Dr Op Sd	Ava (JBB, BL, FWP), Peer (FF, WS)
Rubus argutus Link	W	CP	Woods	Peer (SB, FF, WS, CSW), St Har (CSW)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Rubus cuneifolius Pursh	S	G	Woods	Ava (SB), Peer (FF), St Har (Jahn)
R. hispidus L.	W	G	Fr Mar	reported by O. Brown
R. Baileyanus Britton (*R. villosus* var. *humifusus*)	S	CP	Woods	Peer (FF)
R. flagellaris Willd. (*R. villosus*)	W	G	Sd Du Ho	Peer (SB, FF), St Har (CSW)
Rosa palustris Marsh. (*R. carolina*)	W	CP	Swamp	Ava (JBB), Peer (FF, FWP)
R. virginiana Mill	N	CP	Sd Dune	Peer (FWP), St Har (FF)
R. carolina L. (*R. humilis*)	W	G	Dr Op Sd	Ava (SSVP), Peer (FF, WS)
R. rugosa Thunb.	N	In	Sd Dune	St Har (FF)
Prunus serotina Ehrh.	W	CP	Sd Dune, Woods	Ava (FF, HS), Peer (FF, WS), St Har (FF, CSW)
P. maritima Wang.	W	G	Sd Dune	Ava (HS), Peer (FF)
LEGUMINOSAE				
Cassia Chamaecrista L.	S	CP	Dr Op Sd	Peer (FF, CK, FWP), St Har (FF)
C. nictitans L.	W	CP	Woods edge	Peer (FF)
Baptisia tinctoria (L.) R. Br.	W	G	Dr Op Sd	Peer (FF), St Har (WB)
Trifolium arvense L.	W	In	Dr Op Sd	St Har (FF, WB)
T. pratense L.	W	In	Dr Op Sd	Peer (FF)
T. repens L.	W	In	Dr Op Sd	reported by O. Brown
T. hybridum L.	W	In	Dr Op Sd	reported by O. Brown
T. procumbens L.	W	In	Dr Op Sd	Peer (FF)
T. dubium Sibth.	R	In	Dr Op Sd	Ava (SSVP), Peer (CSW)
Melilotus officinalis (L.) Lam.	W	In	Dr Op Sd	St Har (FF)
M. alba Desr.	W	In	Dr Op Sd	reported by O. Brown
Robinia pseudo-acacia L.	R	In	Dr Op Sd	Peer (FF)
Desmodium Dillenii Darl.	W	G	Wet Sd	reported by O. Brown
D. paniculatum (L.) DC.	W	CP	Swamp edge	Ava (FF), Peer (FWP)
D. obtusum (Muhl.) DC.	S	G	Sd Du Ho	Peer (FWP)
Lespedeza procumbens Michx.	W	CP	Dr Op Sd	Peer (FWP)
L. frutescens (L.) Britton	W	G	Dr Op Sd	Peer (FWP)
L. capitata Michx.	W	G	Dr Op Sd	Peer (FF, WS)
L. striata (Thunb.) H. & A.	S	In	Dr Op Sd	Peer (FWP), St Har (SSVP)
Vicia villosa Roth	W	In	Dr Op Sd	Peer (FF)
Apios tuberosa Moench	W	G	Fr Mar	Ava (HS), Peer (FF, FWP)
Phaseolus polystachyus (L.) BSP.	S	CP	Woods	Ava (HS), Peer (FF, WS)
Strophostyles helvola (L.) Britton	S	CP	Dr Op Sd, Sd Du Ho	Ava (FF, JMF), Peer (FWP, FF)
S. umbellata (Muhl.) Britton	S	CP	Dr Op Sd, Sd Du Ho	Peer (FF)
Amphicarpa Pitcheri T. & G.	S	CP	Woods	reported by O. Brown
LINACEAE				
Linum medium (Planch.) Britton	W	G	Dr Op Sd	Ava (RRD), Peer (FF, WS), St Har (WB, SB, FF, WS)
OXALIDACEAE				
Oxalis stricta L.	W	CP	Woods	Ava (SB, FF), Peer (FF), St Har (CSW)
GERANIACEAE				
Geranium Robertianum L.	N	CP	Woods	Peer (SB, CK, FF, CSW), St Har (Jahn)
POLYGALACEAE				
Polygala cruciata L.	W	G	Fr Mar	reported by O. Brown
P. verticillata L.	W	CP	Dr Op Sd	Ava (FWP), Peer (WS), St Har (SSVP)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
EUPHORBIACEAE				
Euphorbia polygonifolia L.	W	Ma	Sd Dune, Beach	Ava (RRD, FF, JMF), Peer (FF, FWP), St Har (WS)
E. nutans Lag. (*E. Preslii*)	S	CP	Dr Op Sd	Ava (FF)
E. maculata L.	W	CP	Dr Op Sd	reported by O. Brown
ANACARDIACEAE				
Rhus copallina L.	W	CP	Woods	Peer (FF, WS)
R. vernix L.	W	G	Swamp	reported by O. Brown
R. Toxicodendron L.	S	CP	Woods, Sd Dune	Ava (FF, HS), Peer (FF, FWP), St Har (FF)
AQUIFOLIACEAE				
Ilex opaca Ait.	S	G	Woods, Thick	Peer (FF, WS), St Har (FF, WS)
I. verticillata (L.) Gray	W	G	Swamp	reported by O. Brown
CELASTRACEAE				
Euonymus americanus L.	S	CP	Woods	Peer (FF)
Celastrus scandens L.	W	CP	Woods	Ava (FF), Peer (FF, WS), St Har (FF)
ACERACEAE				
Acer rubrum L. var. tridens Wood	S	G	Swamp	Peer (FF, WS), St Har (FF)
BALSAMINACEAE				
Impatiens biflora Walt.	W	CP	Swamp	Ava (FF), Peer (FF), St Har (FF)
VITACEAE				
Psedera quinquefolia (L.) Greene	W	CP	Woods, Sd Dune	Peer (FF, FWP, WS), St Har (FF)
Vitis labrusca L.	W	CP	Woods, Sd Dune	Peer (FF, FWP), St Har (FF)
V. aestivalis Michx.	W	G	Woods, Sd Dune	Peer (SSVP, FWP), St Har (FF)
MALVACEAE				
Kosteletzkya virginica (L.) Presl	S	Ma	Sal Mar	Ava (CK, Witte), Peer (SB, IK), St Har (FF)
Hibiscus Moscheutos L.	S	CP	Sal Mar, Fr Mar	Ava (FF, JMF, IK), Peer (FF, WS), St Har (FF, RRD)
HYPERICACEAE				
Hypericum mutilum L.	W	CP	Swamp	Peer (CK, FWP), St Har (FF)
H. canadense L.	W	G	Wet Sd	Peer (FWP)
H. gentianoides (L.) BSP.	W	G	Dr Op Sd	Peer (FWP)
H. virginicum L.	W	G	Fr Mar	Peer (FF)
CISTACEAE				
Helianthemum canadense (L?) Michx.	W	G	Open Wood	Peer (FF, SSVP)
Hudsonia tomentosa Nutt.	N	Ma	Sd Dune	reported by O. Brown
Lechea minor L.	W	G	Dr Op Sd	reported by O. Brown
L. maritima Leggett	W	Ma	Dr Op Sd	St Har (FF)
L. Leggettii Britton & Hollick	S	G	Dr Op Sd	Peer (FF, FWP)
CACTACEAE				
Opuntia vulgaris Mill.	S	CP	Open Wood	Peer (FF), St Har (FF)
LYTHRACEAE				
Decodon verticillatus (L.) Ell.	W	G	Swamp	reported by O. Brown
MELASTOMACEAE				
Rhexia virginica L.	W	G	Fr Mar	reported by O. Brown
R. mariana L.	S	G	Fr Mar	Peer (FWP)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
ONAGRACEAE				
Ludvigia alternifolia L.	S	G	Fr Mar	Peer (FF)
L. palustris (L.) Ell.	W	CP	Fr Mar	Ava (JBB)
Oenothera biennis L.	W	CP	Sd Dune, Dr Op Sd	Ava (HS), Peer (FF, FWP, WS)
O. humifusa Nutt.	S	Ma	Sd Dune	reported by O. Brown
O. laciniata Hill.	S	G	Dr Op Sd	reported by O. Brown
O. fruticosa L.	W	CP	Dr Op Sd, Thick	Ava (RRD, JMF), Peer (FF, WS), St Har (WB, SB)
O. linearis Michx.	S	G	Dr Op Sd	Peer (FF)
HALORAGIDACEAE				
Proserpinaca palustris L.	W	CP	Fr Mar	Ava (SSVP), Peer (WS)
UMBELLIFERAE				
Eryngium aquaticum L.	S	CP	Fr Mar	Peer (FWP)
Hydrocotyle umbellata L.	S	CP	Fr Mar	Peer (WS)
Ptilimnium capillaceum (Michx.) Raf.	S	CP	Sal Mar, Fr Mar	Ava (FF, JMF, WS, Witte), Peer (FF, CK)
Cicuta maculata L.	N	CP	Swamp	reported by O. Brown
Sium suave Walt. (*S. cicutifolium*)	W	CP	Fr Mar, Swamp	Peer (FF, CK, WS)
Daucus carota L.	W	In	Dr Op Sd	Ava (BL)
CORNACEAE				
Nyssa sylvatica Marsh.	W	G	Swamp	Peer (FF, WS)
ERICACEAE				
Gaylussacia baccata (Wang.) C. Koch	W	G	Woods	Peer (FF, WS)
Vaccinium corymbosum L.	W	G	Woods	Peer (FF)
V. atrococcum (Gray) Heller	W	G	Woods	Peer (FF)
PLUMBAGINACEAE				
Limonium carolinianum (Walt.) Britton	W	Ma	Sal Mar	Ava (FF, JMF, IK), Peer (FF, FWP), St Har (RRD), n Cr I (JMF)
PRIMULACEAE				
Samolus parviflorus Raf. (*S. floribundus*)	W	Ma	Sd Du Ho	Peer (SSVP)
Lysimachia quadrifolia L.	W	CP	Fr Mar	reported by O. Brown
L. terrestris (L.) BSP.	W	G	Swamp	reported by O. Brown
Trientalis americana (Pers.) Pursh	N	G	Woods	Peer (FF, CSW), St Har (McCadden)
EBENACEAE				
Diospyros virginiana L.	S	CP	Swamp, Wet Sd	Ava (FF), Peer (FF, WS)
GENTIANACEAE				
Sabatia stellaris Pursh	S	Ma	Sal Mar	Ava (FF, JMF, Witte), Peer (FF, FWP), St Har (SB, FF)
APOCYNACEAE				
Vinca minor L.	W	In	Woods	Peer (FF)
Apocynum cannabinum L.	W	CP	Thick	St Har (FF)
ASCLEPIADACEAE				
Asclepias tuberosa L.	W	G	Dr Op Sd	Peer (FF)
A. incarnata L. var. pulchra (Ehrh.) Pers.	W	CP	Sal Mar	Ava (FF, JMF), Peer (WS)
A. syriaca L.	W	G	Dr Op Sd	reported by O. Brown
CONVOLVULACEAE				
Convolvulus sepium L.	W	CP	Dr Op Sd	Peer (WS), St Har (FF)
C. s. var. pubescens (Gray) Fernald	W	Ma	Dr Op Sd, Fr Mar	Peer (FF), St Har (SB)
Cuscuta Gronovii Willd.	W	CP	Thick	Peer (FWP)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
BORAGINACEAE				
Myosotis virginica (L.) BSP.	W	CP	Woods	Peer (FF, CK)
VERBENACEAE				
Verbena urticaefolia L.	W	CP	Woods	Peer (FF)
V. hastata L.	W	CP	Sal Mar Bord	Ava (FF, JMF)
Lippia lanceolata Michx.	S	CP	Fr Mar	Ava (JBB)
LABIATAE				
Teucrium canadense L. (*T. c.* var. *littorale*)	W	Ma	Sal Mar, Sd Du Ho	Ava (FF, JMF), Peer (FF, WS), St Har (FF, WB), n Cr I (JMF)
T. c. var. virginicum (L.) Eaton (*T. canadense*)	W	CP	Woods	Peer (FF)
Trichostoma dichotomum L.	W	G	Dr Op Sd	Ava (IK), Peer (HS), St Har (FF)
Salvia lyrata L.	S	CP	Woods	Ava (FF, SSVP), Peer (WS)
Monarda punctata L.	S	G	Open Wood	Peer (FF, FWP)
Lycopus uniflorus Michx.	W	CP	Fr Mar	Peer (FWP)
L. americanus Muhl.	W	CP	Sal Mar Bord	Ava (FF, JMF, HS), Peer (FWP, WS), St Har (FF)
SOLANACEAE				
Solanum dulcamara L.	W	In	Woods	Peer (SSVP)
S. nigrum L.	W	G	Thick	St Har (FF)
Datura Stramonium L.	W	In	Dr Op Sd	reported by O. Brown
D. Tatula L.	S	In	Dr Op Sd	reported by O. Brown
SCROPHULARIACEAE				
Verbascum Thapsus L.	W	In	Dr Op Sd	St Har (WB)
V. Blattaria L.	W	In	Dr Op Sd	Peer (FF)
Linaria canadensis (L.) Dumont	W	G	Dr Op Sd	Ava (Clarke), Peer (FF, FWP), St Har (FF, WB)
Scrophularia lanceolata Pursh	W	CP	Woods	Peer (SB)
Agalinis purpurea (L.) Pennell (*Gerardia purpurea*)	S	CP	Sal Mar Bord	Ava (FF, Kaji, FWP), Peer (FF, FWP), St Har (WB, RRD, FF)
A. maritima Raf. (*Gerardia maritima*)	W	Ma	Sal Mar Bord	Peer (SB, SSVP)
BIGNONIACEAE				
Tecoma radicans (L.) Juss.	S	CP	Woods	Peer (FF, SSVP)
PLANTAGINACEAE				
Plantago major L.	N	Ma	Sal Mar	reported by O. Brown
P. arenaria L.		In	Dr Op Sd	Ava (BL)
P. oliganthos R. & S.	N	Ma	Sal Mar Bord	St Har (FF)
P. lanceolata L.	W	In	Dr Op Sd	Peer (FF, CK)
P. aristata Michx.	W	In	Dr Op Sd	Ava (FF), Peer (FF), St Har (WB, SB)
RUBIACEAE				
Galium Aparine L.	W	CP	Woods	Peer (FF), St Har (FF)
G. pilosum Ait.	W	CP	Open Woods	Ava (BL), Peer (FF), St Har (Jahn)
G. circaezans Michx.	W	CP	Woods	Peer (FF)
G. Claytoni Michx.	W	CP	Fr Mar	Ava (FF, JMF, FWP), Peer (FF, WS), St Har (FF)
G. tinctorium L.	W	CP	Woods	Peer (FF)
G. triflorum Michx.	W	CP	Woods	Peer (FF)
Diodia teres Walt.	S	G	Dr Op Sd	Ava (FF, JMF), Peer (FWP), St Har (FF)
Mitchella repens L.	W	CP	Woods	Peer (FF, WS, Walter)
CAPRIFOLIACEAE				
Lonicera japonica Thunb.	S	In	Woods	Peer (FF), St Har (FF)
L. sempervirens L.	W	CP	Woods	Peer (SB, FF, BL)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Viburnum scabrellum (T. & G.) Chapm.	S	CP	Woods	Ava (FF), Peer (FF), St Har (FF)
V. dentatum L.	W	CP	Woods	Peer (FF)
Sambucus canadensis L.	W	CP	Woods	Peer (FF, WS)
COMPOSITAE				
Eupatorium maculatum L.	W	CP	Fr Mar	reported by O. Brown
E. album L.	S	G	Thick	Peer (FWP)
E. hyssopifolium L.	S	G	Sal Mar Bord, Thick	Ava (JMF), Peer (WS), St Har (FF)
E. verbenaefolium Michx.	S	G	Sd Du Ho	Peer (FF)
E. rotundifolium L. var. ovatum Torr. (*E. pubescens*)	N	G	Sal Mar Bord	Ava (FF, JMF), Peer (FWP, FF)
E. perfoliatum L.	W	CP	Fr Mar	reported by O. Brown
Mikania scandens (L.) Willd.	W	CP	Sal Mar, Swamp	Ava (FF, IK), Peer (SB, SSVP), St Har (FF)
Liatris graminifolia (Walt.) Willd. var. dubia Gray	S	G	Dr Op Sd	Peer (SB, FF)
Solidago bicolor L.	W	G	Woods	Peer (FF, FWP)
S. sempervirens L.	W	Ma	Fr Mar, Sd Dune, Sal Mar	Ava (FF, HS), St Har (FF)
S. suaveolens Schoepf (*S. odora*)	W	G	Woods	Peer (FWP)
S. fistulosa Mill.	S	G	Fr Mar	Peer (FF, WS)
S. rugosa var. aspera (Ait.) Fernald (*S. aspera*)	S	G	Woods	Peer (FWP)
S. nemoralis Ait.	W	G	Thick, Woods	Ava (FF), Peer (FWP), St Har (FF)
S. altissima L.	W	CP	Dr Op Sd	reported by O. Brown
S. graminifolia (L.) Salisb.	W	Ma	Sal Mar Bord	Ava (FF), Peer (FWP)
S. g. var. Nuttallii (Greene) Fernald	W	CP	Sal Mar Bord	St Har (FF)
S. tenuifolia Pursh.	S	G	Sal Mar Bord	Ava (FF, IK), Peer (FWP)
Aster patens Ait.	W	G	Woods	Peer (FWP)
A. undulatus L.	W	G	Woods	Peer (FF)
A. ericoides L.	W	CP	Woods	Peer (FWP)
A. dumosus L.	W	G	Dr Op Sd	reported by O. Brown
A. vimineus Lam.	W	CP	Thick	Ava (FF)
A. lateriflorus (L.) Britton	W	CP	Woods, Thick	Ava (FF), Peer (FF, FWP), St Har (FF)
A. novi-belgii L.	W	G	Swamp edge	Ava (FF, SB), Peer (WS)
A. umbellatus Mill.	W	CP	Fr Mar	reported by O. Brown
A. linariifolius L.	W	G	Woods	Peer (FWP)
A. tenuifolius L.	S	Ma	Sal Mar	Ava (FF, WS), Peer (FWP), St Har (FF)
A. subulatus Michx.	W	Ma	Sal Mar	St Har (FF)
Erigeron ramosus (Walt.) BSP.	W	G	Fr Mar	Peer (FF, Walter)
E. annuus (L.) Pers.	W	CP	Dr Op Sd	reported by O. Brown
E. pusillus Nutt.	S	CP	Dr Op Sd	Ava (FF, IK), Peer (FF)
E. canadensis L.	W	CP	Dr Op Sd	reported by O. Brown
Baccharis halimifolia L.	S	Ma	Sal Mar, Sd Du Ho	Ava (JMF, IK), Peer (FF, FWP), St Har (FF)
Pluchea camphorata (L.) DC.	S	Ma	Sal Mar	Ava (FF, IK, Witte), Peer (SSVP)
Gnaphalium obtusifolium L. (*G. polycephalum*)	W	G	Dr Op Sd, Thick	Ava (IK), Peer (FF), St Har (FF), Cr I (FF, JMF)
G. purpureum L.	W	G	Dr Op Sd	Ava (RRD), Peer (WS), St Har (FF, CSW)
Iva frutescens L. var. oraria (Bartlett) Fern & Grisc. (*I. oraria*)	R	Ma	Sal Mar	Ava (FF, JMF, HS), Peer (SB), St Har (RRD), Cr I (FF)

Species	Range NA	Range NJ	Habitat	Localities and Collectors
Ambrosia artemisiifolia L.	W	G	Dr Op Sd	Ava (FF, JMF), St Har (FF)
Xanthium italicum Moore	W	In	Dr Op Sd	reported by O. Brown
X. echinatum Murr.	W	Ma	Beach, Sd Dune	Peer (FF, FWP), St Har (FF, SSVP)
Helianthus giganteus L.	W	CP	Sal Mar, Fr Mar, Dr Op Sd	Ava (FF, JMF), Peer (FF, SSVP), Cr I (FF, JMF)
Coreopsis lanceolata L.	W	In	Dr Op Sd	Ava (BL), Peer (FF)
Bidens discoidea (T. & G.) Britton	S	CP	Swamp	reported by O. Brown
B. frondosa L.	W	CP	Swamp	St Har (FF)
B. connata Muhl.	N	CP	Fr Mar	Ava (FF), Peer (FWP)
B. bipinnata L.	S	CP	Open Wood	Ava (FF)
B. coronata (L.) Britton	S	G	Fr Mar	Peer (WS), St Har (WS)
Achillea Millefolium L.	W	In	Dr Op Sd, Thick	Peer (FF) St Har (FF, WB)
Chrysanthemum leucanthemum L. var. pinnatifidum Lecoq & Lamotte	W	In	Dr Op Sd	St Har (FF)
Artemisia Stelleriana Bess.	N	Ma	Sd Dune	Ava (FF, JMF)
Erechtites hieracifolia (L.) Raf.	W	G	Thick	Peer (FF, FWP), St Har (FF)
Cirsium spinosissimum (Walt.) Scop.	N	CP	Woods, Dr Op Sd	Ava (Clarke), Peer (FF, WS)
C. discolor (Muhl.) Spreng.	W	CP	Dr Op Sd	Peer (FF)
Krigia virginica (L.) Willd.	W	G	Dr Op Sd	Ava (Clarke, SSVP), Peer (FF)
Hypochaeris radicata L.	R	In	Dr Op Sd	Peer (FF)
Taraxacum officinale Weber	W	In	Dr Op Sd	Peer (CK, FF)
Lactuca canadensis L.[1]	W	CP	Dr Op Sd	Ava (FWP), Peer (FF, FWP)
L. c. var. integrifolia (Bigel.) Gray	S	CP	Dr Op Sd	Peer (WS), St Har (FF)
L. hirsuta Muhl.	W	CP	Dr Op Sd	Ava (JBB), St Har (Jahn)
Prenanthes serpentaria Pursh	S	PB	Woods	Peer (FF, FWP, WS)
P. trifoliolata (Cass.) Fernald	N	CP	Woods	reported by O. Brown
Hieracium venosum L.	W	G	Woods	Peer (WS)
H. scabrum Michx.	W	CP	Woods	reported by O. Brown
H. Gronovii L.[2]	S	G	Dr Op Sd	Ava (JBB), Peer (FF, WS)

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[1] Recorded in Stone (4) as *L. sagittifolia* Ell.

[2] Recorded in Stone (4) as *H. marianum* Willd.

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A STATION FOR HYMENOPHYSA PUBESCENS IN THE EASTERN UNITED STATES

John M. Fogg, Jr.

Late in April, 1936, I first noticed, from the window of a passing train, a colony of cruciferous plants growing on a high embankment along the tracks of the Pennsylvania Railroad a few blocks northwest of the 30th Street Station in Philadelphia. The broad leaves and flat-topped inflorescences strongly suggested *Lepidium Draba*, and as this is not a common introduction in the Philadelphia area the locality was visited a few weeks later for the purpose of collecting material. Although upon this date, May 18, the plant was only in bud, its corymbose inflorescences and oblong, clasping leaves still contributed to its superficial resemblance to *Lepidium Draba*.

On May 27 the plant was in full bloom, its corymbs of whitish flowers forming an attractive sight as seen from the car-window. Specimens collected on this date revealed a few immature silicles, and as these were ovoid rather than flattened it was apparent that the species must belong to some genus other than *Lepidium*. By June 8 its fruits were well developed and the still corymbose heads were crowded with upright, purplish, ovoid to globose, mucronate pods. It was now evident that the plant could be referred to no species included in our current manuals for eastern North America. Comparison with herbarium material, aided by reference to standard Old World treatments, established its identity as *Hymenophysa pubescens* C. A. Mey., a species said to be native to Siberia.

A casual survey of recent literature disclosed the fact that the plant had already twice been recorded from North America. In 1925 Dr. P. C. Standley published a note on its occurrence in Idaho,[1] and the following year Dr. B. A. Walpole called attention to the fact that he had previously (1919) collected and distributed specimens of *Hymenophysa* from Ypsilanti, Michigan.[2]

In an effort to ascertain whether the species had been detected in any of the eastern states, I addressed inquiries to the Field Museum,

[1] Science II, **62**: 509 (1925).
[2] Science II, **63**: 335 (1926).

the Gray Herbarium, the Missouri Botanical Garden, the New York Botanical Garden and the U. S. National Museum. To the authorities of these institutions I am greatly indebted for their kindness in having examined the material in their respective collections and supplying me with all the available records. From the evidence thus accumulated it appears that *Hymenophysa pubescens* has become well established in several of the far western states, e. g., California, Oregon, Washington, Idaho, Wyoming and Colorado, but that it has not before been collected east of Ypsilanti (Washtenaw County), Michigan. The Philadelphia locality, then, is apparently the first to be noted for any of the eastern states.

The plant occupies the crest of a grassy embankment, at 31st and Baring Streets, overlooking the tracks. Although twice cut back and once burnt over by railroad workers, it continues to put up new shoots from its perennial bases. It seems reasonable to suppose that the plant will persist and that in the future it will be reported from additional stations in this area as well as elsewhere on the Atlantic seaboard.

Specimens of five separate collections (Fogg Nos. 10347, 10440, 10527, 11046, 11145) have been deposited in the herbarium of the University of Pennsylvania and duplicates of one or more of these numbers are being distributed to the Philadelphia Botanical Club, the Gray Herbarium, the Missouri Botanical Garden and the U. S. National Museum.

UNIVERSITY OF PENNSYLVANIA.

[*Extracted from* THE JOURNAL OF BOTANY, *November*, 1938.]

A CENTRAL REPOSITORY FOR TYPE-SPECIMENS.

BY F. R. FOSBERG.

TYPE-SPECIMENS are the basis of stability in botanical nomenclature and one of the main factors in taxonomic interpretation. This fact has come to be recognized in the International Rules and is realized, I think, by all taxonomists of any experience whatever. It must also have occurred to many what a catastrophe to the science of botany it would be if one of the major herbaria, or, for that matter, some of the smaller ones, were to be destroyed or seriously damaged in any way. Modern militarism has thoroughly demonstrated its irresponsibility and utter disregard for the welfare of humanity, and its willingness to destroy anything in its path to gain a military objective. The current war in Spain should be given careful consideration by scientists as well as war-experts as a rehearsal for future wars. It has shown conclusively that educational institutions and botanical gardens, in spite of their international character and peaceful inclinations, will receive no special consideration whatever. The state of war is an emergency and all sense of human values is lost.

With world politics in its present unsettled condition and armaments piling up in all of the major nations, it seems time for systematic botanists and others interested in the science to give some thought to the safeguarding of the precious type-specimens on which our nomenclature rests.

A plan calculated to accomplish this end is here presented for the consideration of the botanical public. It is not claimed that this is the best possible plan. It is expected that this or any similar plan will be bitterly opposed by all who place the prestige of their own institutions above the welfare of the science of botany. The suggestion is merely made to stimulate thought and discussion, and with the idea that if it is considered of sufficient importance it may be brought up for discussion at the 1940 international congress at Stockholm. Perhaps by this means something practical and acceptable to all botanists may be evolved and put into operation.

IRREGULAR PAGINATION

Briefly, my suggestion is this. That a modern central herbarium be established for the housing of all types in a locality sufficiently remote from densely populated centres, industrial areas, or points of stragetic importance, to be reasonably safe from destruction in case of any war whatever. This herbarium should be administered by a director and board of regents appointed by the international botanical congress.

If there were a reasonable degree of unanimity among botanists as to the desirability of such an institution, a fund for establishing and endowing it could doubtless be built up by requests to the various research foundations, national research councils, and even to the governments of the important nations. Institutions with a large number of types to be cared for would likely be willing to contribute certain sums for annual running expenses. Gifts from private individuals with an interest in botany would probably not be entirely lacking. An international association of systematic botanists might be organized to promote the welfare of the central herbarium, with small dues to go to maintenance and service.

The administration of this herbarium should, I think, be placed in the hands of a salaried director, appointed by the international congress for a term of five years, subject to renewal at the discretion of the congress. He should be assisted and advised by a council or board of regents of six members, also appointed by the congress, who would have the power, by a two-thirds majority vote, to veto any action of the director that they considered detrimental to the welfare of the institution or the collections housed there.

Facilities should be provided for working and for inexpensive living for visiting botanists who wished to work on the collections housed in the herbarium. Loans of types to accredited institutions would naturally be permitted. Photographs of types would be made at the request of institutions or individuals and distributed at as small a cost as possible. A working collection of specimens other than types and historically important specimens should be maintained, the specimens in so far as possible compared with the types, both for use of resident and visiting botanists and for loan to those interested in special groups. This would prevent shipping damage to more important specimens in all cases where a carefully compared specimen would serve in place of a type. This collection might be built up by the distribution, by exchange, of specimens checked against types, to institutions in all parts of the world. In this way a great service to botany would result at the same time that the central collection was being built up.

The library, necessary in connection with any herbarium, would, in time, naturally become a repository for rare and important works, as well as a very complete collection of all

documents of interest to systematic botanists. A self-supporting photostating or microfilm copying service could be established in connection with the library. Also, a natural outgrowth of this would be a universal bibliographic and abstracting service for systematic botany. A probable result of the maintenance of such a library would be that many of the extensive private libraries (and also herbaria) might be kept together by bequest of the owners, rather than dispersed at their deaths.

Choice of a location for such an institution should only be made after careful deliberation and study of all factors involved, and perhaps only after consultation with military men of several different nations. The place must be remote from any possible military route or battle-ground. Yet it must be reasonably accessible to botanists, without entailing too much travel expense. The climate must not be so extreme in either cold or heat as to discourage botanists from visiting the institution and working there. The region should, if possible, be interesting botanically. Needless to say, deliberation on this subject should be carried on in an atmosphere free as possible from all national or regiona feeling. Switzerland, with its record of hundreds of years of peace, would be a logical choice as a location for such a herbarium if it were not situated in the midst of potentially antagonistic powers. Certain localities in Scandinavia, or perhaps Iceland, would seem likely to remain peaceable, though perhaps the winter climate might be a deterring factor. Sparsely inhabited areas in the southern Rocky Mountain region of the United States might give reasonable safety combined with a moderate climate.

Perhaps the most important question of all, after that of finance, would be that of the status of the types and other historical specimens housed in this herbarium. Of course, some institutions might be willing to give their specimens outright, in the interests of botany, but the majority would probably want to see how the plan worked, before losing control of their specimens. This very reasonable desire might be satisfied in either of two ways. The specimens might be deposited on permanent loan. Or an institution might endow or provide a table and herbarium cases sufficient to house the number of specimens of historical importance in its possession, and make this unit a department of its own herbarium. Its collection would not, however, be maintained in these cases as a unit, as this would be a source of confusion and extra labour for everyone. This would make possible the participation of institutions where the loaning of specimens or of types is forbidden.

Needless to say, the construction and maintenance of such a herbarium could profit by the hundreds of years' experience and the mistakes and successes of all other herbaria combined. Working out of details could be left to the first director and his

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advisory council, who would, naturally, welcome suggestions from experienced persons the world over.

This plan is not presented under any illusion that it is perfect. It seems workable. The benefits that would result to the science of botany would seem to outweigh, by far, any conceivable detriments. If worked out properly it should insure reasonable safety for type-material as long as civilization itself will last, or at least as long as types are necessary to the stability of botanical nomenclature.

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Since the above was written definite news has been received of the deliberate and total destruction of at least two of the largest universities in China, and of some damage by bombs to a third. What happened to their botanical collections, or, for that matter, to the botanists, is not known. Certainly the promising start that the Chinese have made in systematic botany is seriously retarded.

In Europe the most serious crisis since the world war has just been passed. The days during which war seemed practically inevitable must have made many botanists wonder why some such scheme as that presented above was not put into effect long ago. The settlement reached, though peaceable, seems little more than a truce that will enable the powers to build up their military strength still further. No thinking person should be deceived into thinking that a permanent peace has been achieved.

The history of the past year has demonstrated beyond a doubt the desirability of taking some action similar to that outlined above, also the danger entailed by delay and procrastination. By good fortune the herbaria of Great Britain, Prague, Vienna, Paris, and the various German institutions, rich in type-material, escaped exposure to war for the present, but who can predict what may happen next time international politics reaches a boiling point?

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THE LOWER SONORAN IN UTAH

READERS of Professor Cottam's[1] interesting article on the effect of the heavy frost in January, 1937, may be pleased to know that, after all, Utah is in no danger of losing her claim to the Lower Sonoran life zone.

Stimulated by the above-mentioned article and having an opportunity to drive through southwestern Utah in August, 1937, the writer took note of the condition of the Lower Sonoran vegetation, both in the region around St. George and in a considerable area on the alluvial fans west of the Beaver Dam Mountains.

Particular attention was paid to four typical Lower Sonoran plants—creosote bush, *Larrea divaricata;* mesquite, *Prosopis chilensis;* burro weed, *Franseria dumosa;* and desert willow, *Chilopsis linearis.* All these species showed the effects of the frost, but not a single plant was noticed which was completely dead. Numbers of plants of *Larrea* and *Prosopis* had been frozen to the ground, but all these observed were sending forth a vigorous growth of shoots from the base which were already 10 to 50 centimeters tall. The wholesale destruction of the vegetation implied by Professor Cottam's observations of "brown water-soaked cambium layers even at the crown of most shrubs" was not evident along any of the forty or fifty miles of road traveled by the writer in this life zone. Also the bronzing of the junipers in the Upper Sonoran zone noted by Professor Cottam had disappeared, at least in the Beaver Dam Mountains and the regions of Zion and Bryce Canyons.

Interesting was the evident variation in resistance to the frost of plants even of the same species. Some

[1] W. P. Cottam, SCIENCE, 85: 563–564, June 11, 1937.

of the difference in effect was doubtless due to difference in altitude and exposure. The areas at the extreme upper edge of the Lower Sonoran, 900 to 1,000 m in the St. George area and somewhat higher west of the Beaver Dam Mountains, in general suffered more, except where sheltered. But of specimens of *Larrea* growing side by side, with no apparent difference in conditions or size of plant, one would be frozen to the ground, while another might have only the tips of the branches nipped. Every degree of injury excepting actual death was present in a small area of uniform altitude and exposure. However, all plants of *Larrea* had evidently produced far less fruit this year than is usual for this species.

Contrary to Professor Cottam's conclusion that this occurrence "emphasizes the inadequacy of Merriam's theory of zonation in its failure to take into consideration temperature data of the dormant period," these later observations would indicate that, in this instance, at least, the ignoring of the dormant period was quite justified. And a week of sub-zero weather would seem to be quite a severe test. The facts suggest that the life zones are states of equilibrium reached by the vegetation as a result of the action of definite climates over a long period of time, and that they are not likely to be profoundly disturbed by brief intervals of "unusual weather."

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Notes on plants of the Pacific Islands. I.

F. R. FOSBERG

To record miscellaneous and fragmentary observations accumulated while studying and determining plants from various islands of the Pacific it seems worth while to start a series of papers, to appear at irregular intervals as sufficient material accumulates. The first of these is presented here, treating species of the genera *Gouania* (Rhamnaceae), *Diospyros* (Ebenaceae), and *Randia* (Rubiaceae).

Thanks are due to the authorities and staff of the New York Botanical Garden for the privilege of working in the herbarium and for various courtesies extended to me while working there, and also to the authorities of the Bernice Pauahi Bishop Museum, of Honolulu, for the loan of some of the specimens treated in this paper.

GOUANIA

Gouania mangarevica Fosberg, n. sp. Frutex scandens hirsutus vel tomentosus, folia dimorpha piloso-hirsuta supra bullata serrata membranacea vel chartacea, flores in spicas glomerata, spicae maxime 12 cm. longae, bracteoli minuti caduci, flores maxime 3 mm. latae pentamerae, styli 3, discus sub stylis barbatus in lobos triangulares productus.

Liane 20 m. or more long, older parts woody, younger parts hirsute-pilose with tawny spreading hairs, older parts becoming rusty-tomentose; leaves alternate, dimorphic, sparsely hirsute-pilose on both sides, especially beneath on the veins, bullate above, those on young sterile parts of the plants oblong or oblong-ovate, up to 9 cm. long and 4 cm. wide, truncate at base, acuminate at apex, coarsely serrate, especially above, membranous, those on older parts broadly ovate- or elliptic-cordate, obtuse to acuminate, usually shorter than those on sterile branches, as much as 6 cm. wide, chartaceous; tendril-bearing branchlets in the axils of the leaves, these sometimes bearing one or two small leaves, the tendrils slender on young branches but becoming much thickened and woody on the older parts, 2–3 tendrils borne on one branchlet, on older parts these branchlets more robust, sometimes occurring 2 at one node, bearing the inflorescences as well as tendrils. Inflorescence densely yellowish-tomentose, spicate; spikes as much as 12 cm. long, often borne as many as 6 on a branchlet, forming a panicle; glomerules small, subtended by minute caducous bractlets, often scarcely manifest; flowers usually sessile, rarely short-pedicelled, pentamerous, about 3 mm. across when spread out; calyx tomentose externally, its lobes ovate, 1 mm. long, obtuse, glabrous within; petals alternate with the calyx lobes, clawed at the base, about 1 mm. long, the limb suborbicular, saccate, surrounding the upper part of the stamen; stamens opposite and almost equalling the petals; filaments flat, dilated toward the base, inserted beneath the disk between its lobes; anthers almost

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circular, 0.2–0.3 mm. long, the anther sacs terete; disk thin, produced into triangular lobes with strap-shaped apices, opposite the calyx lobes and two-thirds their length, glabrous except for a conspicuous tuft of hair immediately below the styles; styles 3, distinct to the base, divergent, glabrous, fleshy, about 0.7 mm. long; ovary inferior but indistinct in flower; fruit unknown.

GAMBIER ISLANDS, MANGAREVA: South side Mt. Mokoto, upper forest, climbing over bushes and trees, alt. 320 m., June 2, 1934, *St. John 14844* (type) (B); northwest slope Mt. Duff, in woods on low bushes, alt. 110 m., May 24, 1934, *St. John 14488*. (B).

Mangarevan name "*tara koa*" (acc. Theophile, a native informant).

This species seems to resemble most closely the widespread Indo-Malaysian *G. tiliaefolia* and *G. javanica* and also certain similar American species but differs from all in the dimorphic leaves and in the tuft of hair surrounding the styles. This whole group of species of *Gouania* badly needs revision. Perhaps all might be united into one large species with many varieties, as they closely resemble each other. However, certain details in the formation and hairiness of the disk, and especially in the length and degree of union of the styles seem significant. All specimens of *G. tiliaefolia* and *G. javanica* examined except one had very short styles. Most of them had more prominent bracts and less paniculate inflorescences than *G. mangarevica*. More distantly related are *G. vitifolia* of Hawaii, with shorter spikes and quite different leaves, and *G. Richii* of Fiji, with almost glabrous leaves and with the styles united part way up.

Hooker and Arnott (Bot. Beechey Voy. **61**, 1832) report *Gouania dominguensis* L., an American species, from the Society Islands, citing a Lay and Collie collection, and many subsequent authors have credited the species to these islands, presumably on the basis of this report. To my knowledge no other collection has been cited. The authorities at Kew are unable to locate this Lay and Collie specimen, so its identity may not be determined. However, remembering that Hooker and Arnott regarded most of southeastern Polynesia as included in the Society Islands, and since no other *Gouania* has been found in southeastern Polynesia, I think it quite probable that the Lay and Collie specimen belonged to *G. mangarevica*. The expedition spent some time in Mangareva and is known to have collected several other plants there. It is unlikely that it was an American species, unless there was a confusion in labeling, as with several other plants collected by this expedition.

Fruiting specimens are much to be desired.

DIOSPYROS

While working up the Hawaiian species of *Diospyros* I found it necessary to determine the status of the *Maba sandwicensis* reported by Seemann and subsequent authors from Fiji. This did not prove as easy to settle as was expected, and led to a considerable study of various Pacific Ebenaceae, and comparison with available Malaysian and Asiatic material. No attempt was made at a revision of the Pacific species of this group, but some observations were made which seem worth recording. The specimens studied are mainly in the herbaria of the New York Botanical Garden and the Bishop Museum, designated here respectively as (N) and (B). Dates of collections are not cited when collectors' numbers are available.

The recent study of the Malaysian Ebenaceae by R. C. Bakhuizen van den Brink (Bull. Jard. Bot. Buit. ser. 3, XV[1], 1936; XV[2], 1937) treats some of the Pacific species and makes the combinations for several of them under *Diospyros*, but touches upon the extra-Malaysian ones only incidentally. Bakhuizen's reasoning (Gard. Bull. Str. Sett. **7**, pt. 2, 1933) in reducing *Maba* to *Diospyros* seems, in the light of these further studies of Pacific species, to be thoroughly sound, and necessitates the transfer of several Pacific island species from *Maba* to *Diospyros*. Other transfers are doubtless to be made, but material has not been available to determine their exact status.

Diospyros subgen. *Maba* sect. *Ferreola*

Diospyros L. subgen. **Maba** (Forst.) Bakh sect. **Ferreola** (Roxb.) Fosberg, n. comb.

Ferreola Roxb. Pl. Corom. **1**: 35, t. 45, 1795.

Maba sect. *Ferreola* Hiern, Trans. Cambr. Philos. Soc. **12**: 108, 1873.

Diospyros subgen. *Maba* sect. *Forsteria* Bakh. Bull. Jard. Bot. Buit. ser. 3, XV[2]: 50, 1937.

Hiern's name *Ferreola*, based on Roxburgh's genus *Ferreola* (credited to Koenig by Index Kewensis), seems to be the earliest to have been used in the sectional category for this group of species, and so by the rule of priority must replace *Forsteria*. It may be argued that Hiern's section of *Maba* was more inclusive than Bakhuizen's section of *Diospyros*, but the type species of Roxburgh's genus was *Ferreola buxifolia* (*Diospyros ferrea*) which was also included in Hiern's section *Ferreola* and is the principal species of Bakhuizen's section *Forsteria*, though no type species was designated. This seems to make all three nomenclatorially equivalent, and therefore the earliest name must take precedence.

The present studies show that there are at least five distinct species belonging to this section, and a number of additional varieties in the two original species.

Diospyros ferrea (Willd.) Bakh., var. **nandarivatensis** (Gillespie) Fosberg, n. comb.

Maba nandarivatensis Gillespie, Bish. Mus. Bull. **74**: 13, 1930.

Bakhuizen reduces this species outright to *D. ferrea* var. *sandwicensis*, but it seems to me to present differences enough to be maintained as a variety. The leaf-shape is not that of var. *sandwicensis* and the fruit is more nearly that of var. *littorea*. The branched inflorescence (in the staminate plant as shown by *Smith 544* as well as in the pistillate as described by Gillespie) suggests that it may have some relationship with *D. ellipticifolia*, and, indeed, except for the small size of the fruit, might equally well be placed in the latter. It certainly connects the two.

As recognized here, the size of the leaves and fruits varies considerably, but there seems no place to draw a line between them. Those with large leaves tend to have large fruits, but this is not constant.

Specimens examined:

Fiji Is.: Viti Levu, Tholo North Prov., Nandarivatu, *Gillespie 3764* (N); Vanua Levu, Thakaundrove, Natewa Peninsula, Uluingala, *Smith 1982* (N); Vanua Levu, Thakaundrove-Malhuata boundary, crest of Korotini Range, bet. Navitho Pass and Mt. Ndelaikoro, *Smith 544* (N); Vanua Levu, Mbua, southern portion of Seatovo Range, *Smith 1564* (N); Kandavu, hills above Namalata and Ngaloa bays, *Smith 126* (N).

Caroline Is.: Yap, *Kanehira 11840* (N).

Palau Is.: small islands near Korror, *Herre 29* and *36* (N).

The last four collections cited have much larger leaves than the first four.

Diospyros ferrea (Willd.) Bakh., var. **Gillespiei** Fosberg, n. var.

Ramuli glabrati; folia coriacea elliptica vel oblonga subsessilia canaliculata, nervis supra impressis; fructus subsessilis 1.5–2 cm. longus; calix in fructu glaber.

Branches and branchlets gray, beset with prominent lenticels, strigose when very young, but early glabrate; leaves stiff-coriaceous, elliptical to oblong, subsessile, canaliculate, the veins prominently impressed above, the blade up to 8 cm. long and 3.5 cm. wide, glossy, minutely appressed puberulent when very young, but soon becoming glabrate; flowers not seen; fruits subsessile, cylindric to ellipsoidal, sparsely appressed hirtellous, 1.5–2 cm. long, tipped with the persistent style; fruiting calyx cupulate, 7–8 mm. across, its lobes rounded to obtuse, low, glabrous inside, glabrous or almost so outside.

Fiji Is.: Viti Levu, Naitasiri Province, woods near road beyond Tamavia village, 7½ mi. from Suva, alt. 150 m., *Gillespie 2450* (B, N); same loc., *Gillespie 2146* (B), (type).

Referred by Gillespie to *Maba sandwicensis*, by A. C. Smith to *Maba buxifolia*, and by Bakhuizen to *Diospyros ferrea* var. *littorea*.

Diospyros ferrea (Willd.) Bakh., var. **subimpressa** Fosberg, n. var.

Ramuli glabrati; folia coriacea elliptica glabrata, nervis supra subimpressis; fructus ovoideus vel ovalis strigosus, calix in fructu planus vel reflexus strigosus.

Branches brownish, beset with prominent lenticels, sparsely strigose when young, glabrate; leaves stiffly coriaceous, elliptical, acute at base, rounded-acute at apex, sparsely strigose beneath when young, glabrate, the veins somewhat impressed above, the petiole about 2.5 mm. long; flowers not seen; fruits solitary on peduncles 2 mm. or less long, ovoid to broadly oval, up to 15 mm. long and 13 mm. wide, tipped with the persistent style, rather persistently strigose; fruiting calyx flat or with lobes reflexed, about 7 mm. wide, the lobes low, obtuse, strigose inside and outside.

Fiji Is.: Viti Levu, Rewa Province, woods near summit of Korombamba mountain, *Gillespie 2324* (B), (type).

Very close to var. *Gillespiei*, differing chiefly in the shape of the fruit and in the less conspicuously impressed veins. Referred to *Maba sandwicensis* by Gillespie.

Diospyros ferrea (Willd.) Bakh., var. **savaiiensis** (Christ.) Fosberg, n. comb.

Maba savaiiensis Christophersen, Bish. Mus. Bull. **128**: 173, 1935.

Characterized by coriaceous leaves, reticulate on both sides, abruptly contracted, then shortly attenuate into a petiole, blade ovate-acute, fruiting calyx reflexed, appressed puberulent inside and out, and fruit appressed puberulent. Plant otherwise glabrous or very early glabrate.

Samoa: Savaii, Falealupo-Fagalele, *Christophersen 3328* (N).

Diospyros ferrea (Willd.) Bakh., var. **angustifolia** (Miq.) Bakh.

Schlechter 19205 from "Walder bei Alexishafen, Kaiser-Wilhelmsland" (N) is, so far as I know, the first record of this variety from New Guinea. It agrees well with material of this variety (*Thwaites; DeSilva 43* from Ceylon (N), cited by Bakhuizen. The other two specimens cited by him, *Stocks* from Malabar Concan (N) and *Kajewski 932* from the New Hebrides (N) do not agree so well, and probably do not belong to this variety.

Diospyros ellipticifolia (Stokes) Bakh., var. **elliptica** (Forst.) Fosberg, n. comb.

Maba elliptica Forst. Char. Gen. Pl. 122, *t. 61*, 1776.

Ferreola ellipticifolia Stokes, Mat. Med. 535, 1812.

This, the typical form with elliptic obtuse leaves, dull and venulose above, may be reported, I think for the first time, from Niuafou, (*Jaggar*, Oct. 1930, (B)).

This variety is widespread from Samoa to Malaysia and southeastern

Asia. Specimens were examined from Samoa, New Caledonia, and Amboina (all N). Forster's original material came from Tonga.

Diospyros ellipticifolia (Stokes) Bakh., var. **iridea** Fosberg, n. var.

Maba aff. *elliptica* Christophersen, Bish. Mus. Bull. **128:** 173, 1935.

Arbor, ramuli aureo-sericei glabrati; folia lanceolata; flores paniculatae; fructus 23–25 mm. longus leviter umbonatus; calix in fructu cupulatus glabratus.

Tree 6 m. tall, branches terete, grayish brown, rather smooth; leaves not congested; young parts at first golden-sericeous, very early glabrate; leaves lanceolate, slightly acuminate, blunt, up to 11 cm. long and 3 cm. wide, chartaceous, glossy, drying an almost iridescent gray-brown, the petiole 3–5 mm. long; staminate flowers in loose 4–5 flowered panicles about 10–13 mm. long, panicle and calyx densely sericeous; calyx tube 2 mm. long, lobes 1 mm., acuminate; corolla tube cylindrical, 4 mm. long, 1 mm. wide, sericeous just beneath the lobes, otherwise glabrous, lobes ovate, 1 mm. long, sericeous; pistillate flowers not known; fruit on a peduncle 1 cm. long, red when ripe, yellow when immature, 23–25 mm. long, 15 mm. wide, slightly umbonate at apex; fruiting calyx glabrate, cupulate, 7–8 mm. across, lobes obtuse.

SAMOA: SAVAII, near Tufutafoe, alt. 10 m., *Christophersen 2272* (B, N), *3310* (B, N) (type); (type sheet B). No. *2272* is in flower and no. *3310* in fruit.

Differs from var. *elliptica* in the shiny thin lanceolate leaves, light colored smooth twigs, acuminate calyx lobes, pedicellate flowers, much smaller calyx and corolla, and red fruit.

Diospyros lateriflora (Hiern) Bakh.

This species may be reported from Uvea on the basis of *Burrows W. 23* "inland, edge of forest, el. 15 m." (B). Burrows gives the aboriginal name as *mapa*. This specimen was labeled *Maba sandwicensis* in the herbarium. *Parks, 16291* from Liku Terrace, Eua, Tonga Is. (N) and a collection from Tonga, without island or locality, *McKern 99* (B), also belong to this species.

Some doubt was expressed by Bakhuizen in assigning this species to his section *Forsteria*. The material at hand shows that the ovary is definitely 3-celled, the cells 2-ovuled, the ovary hairy, the style trifid, and staminodia absent; seeds were not available, so the endosperm could not be examined. There seems little doubt that it belongs in this section, here called *Ferreola*.

Diospyros globosa (A. C. Smith) Fosberg, n. comb.

Maba globosa A. C. Smith, Bish. Mus. Bull. **141:** 121, 1936.

This is close to *D. lateriflora*, as Smith says. It may eventually prove only a variety, if further collecting should reveal intergrades, as the differences are no greater than the extremes in *D. ferrea* and *D. ellipticifolia*.

Material now available does not justify any change in its status, other than the generic transfer.

The fruits on *Smith 1242* are not mature and would probably approach in size those of *D. lateriflora* at maturity.

FIJI IS.: Kambara, *Smith 1241* (isotype), *1242* (both N).

Diospyros Christophersenii Fosberg, n. name

Maba samoensis Hiern, Jour. Bot. **15:** 99, *pl. 186*, 1877.

Not *Diospyros samoensis* Gray.

Amply characterized by Hiern. Known only in fruit. Probably to be placed in sect. *Ferreola*, though in absence of flowering specimens this is uncertain.

SAMOA: Savaii, Siuvao-Auala, *Christophersen 3379* (N).

Diospyros subg. *Maba* sect. *Cupulifera*

Diospyros L. subgen. **Maba** (Forst.) Bakh. sect. **Cupulifera** Fosberg, n. sect.

Calix in fructu insigniter accrescens, fructum usque ad medium cingens.

Fruiting calyx extremely accrescent, woody, surrounding the lower half of the fruit; ovary trilocular; fruit and calyx copiously rusty pubescent.

Diospyros rufa, characterized by its peculiar acorn-like fruit, was placed in *Maba* sect. *Ferreola* by Hiern, and would go, according to Bakhuizen's key, into sect. *Forsteria*, but seems to have little in common with the rest of the members of the section. Those who follow a narrow genus concept would doubtless refuse to include them in the same genus. So far as I know, the type species, *D. rufa*, in the sense accepted below, is the only species in the section described above.

Diospyros rufa (Labill.) Fosberg, n. comb.

Maba rufa Labill. Sert. Austr. Caled. 33, t. 36, 1824.

Maba sericocarpa F. Muell. Fragm. **5:** 164, 1866.

Maba cupulosa F. Muell. Fragm. **5:** 164, 1866.

Diospyros sericocarpa F. Muell. Austr. Veg. in Intercol. Exh. Ess. 1866–67: 35, 1867.

Diospyros cupulosa F. Muell. Austr. Veg. in Intercol. Exh. Ess. 1866–67: 35, 1867.

Maba yaouhensis Schlecht., Engl. Bot. Jahrb. **39:** 226, 1906.

?*Maba parviflora* Schlecht., Engl. Bot. Jahrb. **39:** 226, 1906.

Material of this species from New Caledonia has generally broader and more obtuse leaves than that from Australia.

Schlechter's species cannot, on the basis of material available, be maintained as specifically distinct. The small flowers of the material upon which *M. parviflora* was based suggest that if more material were available it might prove to be a good variety.

RANDIA

Randia cochinchinensis (Lour.) Merr. Tr. Am. Phil. Soc. **24** (2): 365, 1935.

Aidia cochinchinensis Lour. Fl. Cochinch. **143**: 1790.

Stylocoryne coffeoides Gray, Proc. Am. Acad. **4**: 309, 1860 (excl. syn.).

Randia coffeoides (Gray) B. & H. Gen. Pl. **2**: 88, 1873.

Randia Graeffei Reineke, Engl. Bot. Jahrb. **25**: 683, 1898.

Full synonymy in Merrill, Tr. Am. Phil. Soc. **24**(2): 365, 1935.

The widespread Pacific *Randia coffeoides* does not seem to differ significantly from the above tropical Asiatic and Malaysian species. Plants from Polynesia and Micronesia tend to have the buds slightly bent and sharper at the apex than most Asiatic specimens, but some, even from Ceylon, cannot be distinguished from those from the Pacific. Neither can I distinguish *R. Graeffei* on the basis of the description and a number of collections from Samoa. I have not seen the type. It is considered by Kanehira (Bot. Mag. Tokyo **45**: 349, 1931) to be a synonym of *R. racemosa* (Cav.) F.-Vill., itself a synonym of *R. cochinchinensis*.

Stylocoryne coffeoides Gray was so inadequately characterized that it is doubtful whether it could be considered validly published. Neither specimens nor localities were cited, and both synonyms given by Gray have been shown by Seemann (Fl. Vit. 123) to belong to other genera altogether. The U. S. Exploring Expedition specimens and a collection by Harvey labeled by Gray are the only means of positive identification of the species. Duplicates of these that I have seen in the herbarium of the New York Botanical Garden are certainly *R. cochinchinensis*. Seemann (l.c.) considered these identical with *Stylocoryne racemosa* Cav. which Gray definitely excluded from his species. Merrill (l.c.) considers *S. racemosa* Cav., of the Philippines, to be a synonym of *R. cochinchinensis*. This seems to add the weight of Seemann's opinion to my reduction of *R. coffeoides* to *R. cochinchinensis*.

The range of the species in the Pacific extends from the Society Islands westward through most of the major island groups to Malaysia, and beyond through tropical Asia. It has been collected from most of the high island groups including the Societies, Samoa, Wallis, Fiji, Tonga, New Hebrides, Solomons, Marianas, Carolines, Palau, etc. It does not occur in the Hawaiian Islands, and has not been collected in the Cook Islands or the Marquesas, and, strangely enough, apparently not in New Caledonia. It may here be reported from the island of Niuafou on the basis of a specimen in the Bishop Museum collected by *T. Jaggar*, Oct. 1930.

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[*Extracted from* THE JOURNAL OF BOTANY, *August*, 1938.]

TWO QUEENSLAND IXORAS.

BY F. RAYMOND FOSBERG.

DURING work on the Rubiaceae collected by the Mangarevan Expedition of the Bishop Museum (see Fosberg, Bishop Mus. Occ. Pap. xiii. no. 19, 1937) the question arose of which of the two species named *Ixora triflora* had priority. Both were said to have been published in 1866. The fascicle of Seemann's 'Flora Vitiensis' in which *Ixora triflora* (Forst.) Seem. was published is dated April 2, 1866. Inquiry at Kew resulted in the information that volume iii. of Bentham's 'Flora Australiensis,' in which

Ixora triflora R. Br. was published, though dated 1866, really did not appear until January 5, 1867, giving Seemann's species clear priority.

Since *Ixora triflora* R. Br. is closely related to other species of *Ixora* sect. *Phyleilema* I thought it best, before renaming it, to examine any available material to make certain that it is distinct. Several sheets were borrowed from the herbaria of the Botanic Garden, Brisbane, the Arnold Arboretum, and the Bishop Museum, Honolulu. Thanks are here expressed to Dr. C. T. White, Dr. E. D. Merrill, and Mr. E. H. Bryan, in charge of these collections respectively, for the privilege of examining this material. In all, three collections were examined, with several sheets of two of them, which had been credited to this species.

These specimens were found to represent two entirely distinct species, the *I. triflora* of R. Brown, here renamed *I. queenslandica*, and another species so different that it is a matter of some doubt whether it is correctly placed in the genus *Ixora*. Original descriptions of both are presented below to facilitate comparison.

Ixora queenslandica Fosberg, nom. nov.

Ixora triflora R. Br. in Benth. Fl. Austr. iii. 416, Jan. 5, 1867 (in part).

Non *Ixora triflora* (Forst.) Seem. Fl. Vit. 133, April 2, 1866.

Small tree, branchlets glabrous, slender, internodes up to 2·5 cm. long; leaves obovate to elliptic, blunt-obtuse at apex, cuneate at base, up to 7 cm. long and 3·5 cm. wide, thin-coriaceous, glabrous, petiole 4–6 mm. long; stipules only slightly connate, ovate, firm but with a noticeable thin wing at each side, strongly carinate toward the apex, not so near the base, beak very strong and slightly incurved, sharp or blunt, the whole stipule up to 4 mm. long, caducous; cyme of 3 sessile or subsessile flowers, the central one with a pedicel about 1 mm. long, peduncles up to 22 mm. long; bracts almost orbicular, cordate, apex rounded, petiole less than 1 mm. long, but lower 5 mm. of mid-rib thickened (as in *I. Setchellii*), bracts up to 2 cm. long and wide; hypanthium glabrous, 1·5 mm. long; calyx about 1 mm. long, rather sharply but remotely denticulate, slightly ciliate; corolla-tube 10–13 mm. long, or less in stunted cymes, 0·8 mm. thick, the four lobes up to 8 mm. long, 2 mm. wide, oblong-lanceolate, acute, somewhat contorted in bud; anthers up to 4 mm. long, linear-lanceolate, acuminate, sagittate at base, attached in the sinuses of the corolla, filament less than 1·5 mm. long; style filiform, up to 18 mm. long, exserted 4–5 mm., upper 1·5–2 mm. thickened and bifid.

Moore (Journ. Bot. lxiv. 216, 1926) gives the following information not available on the specimens before me: "corolla white; fruit subglobular, 6×5 mm."

Specimens seen: Queensland, Percy Is., Pine Island, scrub, March 1906, *Tryon* (Brisbane Herb.; Arn. Arb.); another specimen, fragmentary and without data, determined in F. M. Bailey's writing (sec. C. T. White) (Brisbane Herb.).

Although I was unable to borrow the type material of Brown's species I think there is little doubt that the Tryon specimens are identical with it, as they were compared by Moore (see Journ. Bot. lxiv. 216) and considered identical.

As originally described *I. triflora* R. Br. represented a confusion of the *Ixora* material with specimens of *Diplospora ixorioides*. This confusion was pointed out by Spencer Moore (*loc. cit.*), and the species as here renamed includes only *I. triflora* R. Br. as limited by him, not in the original sense as published by Bentham.

It is difficult with the present lack of material of most of the species of *Ixora* sect. *Phyleilema* to make any suggestion as to the relationship of *I. queenslandica* with any of the other species. Certain similarities with *I. Setchellii* Fosberg are probably merely the result of parallel development. When more complete collections are available from Melanesia and the Papuan area relationships in this section of *Ixora* may become more obvious.

Among collections distributed by the Arnold Arboretum is a plant collected by L. J. Brass in Queensland, labelled "*Ixora triflora* R. Br." Dr. C. T. White suggested that this plant might represent a different variety. Open flowers and fruits are not present on the specimens, but careful dissection and examination of the flower parts shows features possessed by no other *Ixora* known to me. There is some doubt that it even belongs in this genus, but in the absence of fruit it would be unwise to set up a new genus for it, and the aspect and most other characters are those of an *Ixora*.

Ixora biflora Fosberg, sp. nov.

Frutex, folia elliptica vel oblonga vel obovata acuminata, cyma terminalis bracteata reducta ad flores solitarias vel geminates, hypanthium glabrum tarde hirtellum, corolla glabra 4-loba, antheres lata oblonga 1·2 mm. longa in alabastris dehiscentes.

"Shrub about 4 ft. tall," branchlets cylindrical, glabrous, woody almost to the tips, internodes up to 3·5 cm. long, frequently under 1 cm. on branchlets; leaves oblong to elliptical or slightly obovate, acuminate, "thin and soft, paler on under surface," up to 9 cm. long and 4 cm. wide, glabrous, base contracted, petiole 6 mm. long; stipules broadly ovate, not carinate except at apex which is prolonged into a sharp beak, the whole 4 mm. long; ultimate branchlets much condensed with imbricate bracts and their stipules, producing an appearance of terminal leafy buds, from which the corollas project singly or in pairs between a pair of leaf-like bracts, ovate, acuminate at apex, 1–1·5 cm. long, up to 8 mm. wide, obtuse at base, with a slight winged petiole less than 1 mm. long, the imbricate bracts sur-

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rounding the flowers thin, pale green, elliptical, sharply acuminate, about 1 cm. long, 5 mm. wide ; flowers terminal, single or paired, sessile ; hypanthium about 1·5 mm long, glabrous at first, but after shedding of corolla becoming woolly-hirtellous ; calyx about 1·5 mm. long, membranous, cup-shaped, irregularly lobed, minutely fimbriate-ciliate, otherwise glabrous ; corolla white, glabrous, not known except from buds almost ready to open, typically *Ixora*-like but not or only slightly twisted, evidently nodding in bud, tube up to 14 mm. long, enlarged slightly at base and slightly funnel-form at throat, 0·8 mm. thick, lobes 6 mm. long, imbricate in bud, but not or only slightly contorted ; anthers broadly oblong, 1·2 mm. long, attached in the sinuses of the corolla, dehiscing, evidently introrsely, while the bud is still closed, showering the inside with pollen ; style completely undeveloped in the buds at this stage, represented only by a small conical prominence on the disk, but evidently deciduous, as it is represented only by a circular scar on an ovary which had just shed its corolla, the disk on this a raised somewhat irregular ring ; ovary of buds too young to make out anything with certainty, even with thin sections, the older ones attacked and destroyed internally by insects ; fruits unknown.

Specimens seen : North Queensland, slopes of Mt. Demi, rain-forests, alt. 2000 ft., Feb. 6, 1932, *L. J. Brass* 80 (type in Brisbane Herb., duplicates in Arnold Arb. and Bishop Mus.).

The unusual feature of the ovary becoming woolly after the shedding of the corolla is evident from the presence of glabrous young ovaries and woolly older ones on the Bishop Museum specimen.

The only plant that I can find mentioned which could be related at all closely to this is a little-known species from New Guinea, *Ixora coffeoides* Valeton, of which only the fruit and very young buds are known. It is excluded from *Ixora* by Bremekamp (Bull. Jard. Bot. Buitenz. ser. iii. xiv. 348, 1937), who suggests that it may come nearer to *Diplospora*. The latter genus, however, so far as I know, never has a sclerified endocarp, which *Ixora coffeoides* has, in common with other species of *Ixora*. The lack of a concavity on the inner side of the pyrene is the most obvious difference which would exclude it from *Ixora*. Features of *Ixora biflora* different from other *Ixora* species are the short and broad anthers and the extreme retardation of development of the style. A delayed opening of the style-lobes to expose the stigmatic surfaces is a feature of the whole tribe Ixoreae. Also there is a vague suggestion in the sections of the very young ovaries available of more than one ovule in a cell, but the material is too young for the point to be determined with certainty. It would seem best to retain these two species in *Ixora* until flowers of *I. coffeoides* and fruits of *I. biflora* are available. Then their relationship and generic affinities may be determined more satisfactorily. At present neither can be placed in any of Bremekamp's subgenera.

The sheet of *I. biflora* in the Brisbane herbarium is designated the type, as it has the most flowers, though the more mature ovaries are on that in the Bishop Museum. The sheets in the Bishop Museum and the Arnold Arboretum may be considered isotypes.

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ADDITIONAL NOTE ON QUEENSLAND IXORAS.

By F. R. FOSBERG.

AFTER the manuscript of the article on "Two Queensland Ixoras" (Journ. Bot. lxxvi. 233–237, 1938) was submitted for publication, Mr. C. T. White was kind enough to send for my examination two additional recent collections of *Ixora biflora* with the suggestion that one of them might represent a new variety. This turned out to be so, and the variety *Fleckeri* is described below and also a varietal name is given to the typical form.

One of the collections, Mossman Gorge, Queensland, June 20, 1937, *Flecker* 3509 seems identical with the type of the species, and, fortunately, bears a mature fruit, from which the following may be added to the description of the species: fruit "bright scarlet," somewhat flattened, slightly grooved on the flat sides, bearing a large calyx scar at apex, subsessile, 11 mm. high, 13 mm. wide, 6–7 mm. thick when dry, surface when dry rugulose-papillose, subtended by two or more persistent bracts, these not, or only slightly, accrescent.

IXORA BIFLORA Fosberg var. **typica** Fosberg, nom. nov.

This is the typical form of the species as described on p. 235.

IXORA BIFLORA Fosberg var. **Fleckeri** Fosberg, var. nov.

Folia oblongo-lanceolata acuminata, hypanthium persistens glabra, calix valde 4-dentatus.

Differing from var. *typica* in having oblong-lanceolate acuminate leaves up to 9 cm. long and 2·5 cm. wide; hypanthium not becoming hirtellous, but remaining glabrous, calyx margin not fimbriate-ciliate, but glabrous and prominently 4-dentate; fruit maturing later, those on specimen young and green, about the size of small peas.

Specimen seen: Queensland, Mossman Gorge, jungle at intake, June 20, 1937, *Flecker* 3521 (type, Brisbane Herb.).

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THE EFFECT OF LIGHT ON THE FRUITING OF MYXOMYCETES [1]

William D. Gray

Although the myxomycetes are unique in that they unite in themselves a number of characteristics of both animals and plants, they have been investigated by relatively few biologists. As a result of this neglect there are many gaps in our knowledge of their simpler physiological processes, and a search through the literature reveals the fact that little is known of them except perhaps the taxonomy within the group. This situation cannot be attributed to a lack of interest or inclination on the part of biologists, although it might be explained to some extent by the rather uncertain position of the myxomycetes. One reason for so little being known is probably the fact that only a few species have been cultured. Our present knowledge of the biology of this group has been obtained from the few forms which can be easily cultured in the laboratory and from observations made on species collected in their vegetative phase. The limitations of this latter procedure are obvious: No matter how assiduous a worker may be, there are many species which he may never see in the vegetative state; in fact, the plasmodia of only about one-third of the described species have ever been seen and recorded. The problem that immediately confronts us then is that of culturing species of myxomycetes which have never been grown in the laboratory.

[1] Received for publication April 25, 1938.
The writer is indebted to Dr. Harlan H. York and Dr. Conway Zirkle for helpful criticisms in the preparation of the manuscript.

Certain species have been cultivated successfully, but these form a very small percentage of the total. *Physarum polycephalum* Schw. can be most satisfactorily cultured at present. Howard (1931) first successfully cultured this species in the laboratory, and further improvements by Camp (1936) made the cultivation of *P. polycephalum* much simpler than that of most fungi. Skupienski (1920, 1928) has cultured both *Didymium difforme* (Pers.) Duby and *D. nigripes* (Link.) Fr. on potato and carrot decoction agar, and Lister (1888) has described the cultivation of *Badhamia utricularis* (Bull.) Berk. on the sporophores of various agarics. The limitation of Lister's method is obviously the fact that such sporophores are not available at all times of the year. Klebs (1900) cultured *D. difforme* and *D. effusum* (synonym of *D. squamulosum* (Alb. & Schw.) Fr.), and Cayley (1929) cultured *D. nigripes* and *D. xanthopus* (Ditm.) Fr., in addition to the two species of *Didymium* used by Klebs, on Knop agar. A few other forms have also been cultured, but, considering the fact that there are about four hundred species of myxomycetes, it is clear that comparatively few have been grown in the laboratory.

Many attempts of the writer to cultivate various species (largely of the order Physarales) from living plasmodia, collected in their natural habitats, have generally failed because of the rapid formation of fruiting bodies by the plasmodia when brought into the laboratory, despite efforts to simulate conditions

under which they were growing in nature. Such results lead to the suggestion, already advanced by Cayley (1929) and Seifriz and Russell (1936), that there is a definite rhythmical fruiting exhibited by myxomycetes. Cayley found that *Didymium difforme* possesses a much shorter vegetative phase than either *D. nigripes* or *D. squamulosum* but stated that the factors causing plasmodia to form fruiting bodies are "still a complete mystery" (p. 234). If such rhythmical fruiting does exist, it is highly possible that the species observed by the writer may possess rhythms of relatively short duration in so far as the vegetative phase is concerned. Unless this rhythm is so innate as to be unalterable, it should be possible, by controlling various environmental factors, to vary the rhythm one way or the other so that plasmodia would vegetate for a longer or shorter period than normal. Thus it is entirely possible that a larger number of species might be brought into and maintained in cultivation if methods of prolonging the vegetative phase might be found. Of course, the problem of nutrition must also be considered, but even if the nutritional requirements of a species be known, it would be an extremely arduous task to maintain it in culture if it remained in the vegetative state for only a short period. Undoubtedly, a plasmodium about to form fruiting bodies differs fundamentally from one which is beginning its vegetative existence, even though the exact nature of the difference is not known. If the change from vegetative to fruiting phase is due to chemical factors, physical factors, or a combination of both, it should be possible to hasten or delay it. As mentioned above, the factor of nutritional requirements is an important one, and no attempt is made to minimize it; however, this can undoubtedly be ascertained by experimentation with various species.

The present series of experiments are concerned with the influence of light upon the length of time required to develop fruiting bodies in various species of myxomycetes. There is a possibility that these findings may have a bearing upon the culture of other species as well as contribute to our knowledge of the physiology of the group. It is quite probable that temperature, moisture, texture of substratum, nutrient materials, and degree of acidity or alkalinity of the medium are also important, but in the present work the influence of light alone is described.

Almost no investigations have been made of the effects of light upon myxomycete plasmodia. The most extensive work in this field was that Baranetzki (1876), who, however, limited himself almost entirely to studies of the heliotropic responses of plasmodia of *Fuligo septica* (L.) Weber and the interrelationships of heliotropic and geotropic responses. The remaining references in the literature are merely scattered observations made by various workers in the course of other studies; these observations will be referred to in the discussions of the experiments conducted by the writer.

Materials and methods.—With the exception of plasmodia of *Physarum polycephalum* Schw., which were derived from sclerotia, plasmodia of all species cultured in the laboratory were obtained from spore cultures. Since there is not enough surface moisture on agar slants to insure good spore germination, cultures were made by adding 1 cc. of sterile distilled water to each slant; spores were then sown directly in the water. Such cultures were placed in the dark, as various workers have shown that light destroys swarm cells. When plasmodia developed, they were maintained in culture by means of plasmodial transplants to fresh nutrient media. Three culture methods were used: (1) the moist chamber method developed by Camp (fig. 7), (2) Petri plate culture (fig. 13), and (3) beaker culture (fig. 9). With *P. polycephalum*, all three methods were employed, whereas only Petri dish cultures were made for *P. tenerum* Rex, *P. compressum* Alb. & Schw., *Didymium xanthopus* (Ditm.), Fr., *Hemitrichia vesparium* (Batsch) Macbr., *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

In moist chamber cultures of *P. polycephalum*, the plasmodia were fed every twenty-four hours by sprinkling ground-up rolled oats directly on them; in both beaker and Petri dish cultures of this same species, three per cent rolled oat agar was used. *P. tenerum*, *P. compressum*, *F. septica*, and *H. vesparium* were grown on two and five-tenths per cent corn decoction agar, adjusted to pH 5.4–5.6; *D. xanthopus* was grown on five per cent carrot decoction agar; *L. fragilis* was grown on both corn decoction agar and three per cent hay infusion, one per cent dextrose agar.

In experiments involving light intensity, the cultures were placed under light bulbs of various wattage, the bulbs being suspended three feet above the open top boxes in which the cultures were placed. All experiments involving such artificial light were conducted in a room in which the temperature varied from 23° to 26°C. Experiments involving direct sunlight were conducted in a greenhouse where the temperature remained at 21°C. in the daytime, dropping to as low as 15°C. at night.

Species herein considered are divided into three groups, based on plasmodial color: yellow pigmented, non-pigmented, and variable plasmodial types, which ranged from white to cream-colored and various light shades of yellow.

Yellow-pigmented plasmodial types.—This group includes the species whose plasmodia are normally yellow at all times, except for the period immediately previous to the formation of fruiting bodies. In this group are included *Physarum polycephalum* Schw., *P. tenerum* Rex., *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

Physarum polycephalum Schw.—It was possible to conduct many more experiments with this species than with any of the others, because of the ease with which it can be cultured, the rapidity of its growth, and the subsequent formation of relatively large amounts of protoplasm. As has been previously mentioned, all three types of culture methods were utilized, but the majority of experiments was conducted with moist chamber cultures which were fed equal amounts (0.5 gm.) of pulverized rolled oats every twenty-four hours. It was soon discovered that the plasmodia could not be kept in direct sunlight, although whether the destruction was due to the intensity or quality of the light is not yet known; possibly both factors were concerned.

Preliminary experiments revealed that, except in a few cases, plasmodial cultures of the beaker or Petri dish type would not form fruiting bodies in the complete absence of light if they were inoculated from stock cultures that had been maintained in the dark. This was also found to be true for moist chamber cultures that were fed every twenty-four hours. Several hundred cultures of this type were set up in the dark at various times and only three or four fruited, while check cultures grown in artificial or diffuse natural light nearly always formed sporangia. Those few cultures that did fruit in the dark passed a considerable time (15 days or over) in the vegetative stage. In cultures inoculated from stock plasmodia maintained in strong natural light, a delayed effect was frequently shown, for cultures of this type often fruited in the complete absence of light. Sixty cultures were inoculated from stock plasmodia which had been kept in the light; of these, seventeen (28 per cent) produced sporangia in the dark. In order to overcome any delayed effect, all subsequent experiments were conducted with cultures inoculated from stock plasmodia maintained in the dark. For the sake of uniformity, five-day old stock cultures were arbitrarily chosen as sources of inocula.

Plasmodia grown in light were always a paler yellow than those kept in the dark. Hofmeister (1867, p. 21) stated that plasmodia of *Fuligo septica* (L.) Weber which were kept in the dark were paler than those grown in the light; however, Baranetzki (1876), working with the same species, stated that the reverse was true. The observations of the writer on yellow-pigmented plasmodia tend to confirm the findings of Baranetzki rather than those of Hofmeister. It was also noted in *P. polycephalum* that plasmodia grown in the dark showed a tendency to spread, whereas the protoplasm of plasmodia grown in light showed a decided tendency to form clumps and large, swollen plasmodial "veins." These two types of plasmodial configurations are shown in figures 4 and 5.

The finding that light was nearly always essential for the fruiting of this species, under the conditions outlined, naturally suggested that the intensity of light might condition the time of fructification, and it was subsequently found that a definite correlation exists between light intensity and duration of vegetative phase. Over a period of two years moist chamber cultures were grown in open top boxes under 60, 100, 150, 200, and 400 (two 200's) watt bulbs, suspended three feet above the cultures, and it was found that under each type of light (with the exception of the most intense) a rather definite fruiting rhythm was exhibited. The following list summarizes these data:

Cultures under	60	watt light fruited in	12	days
"	"	100	"	"	"	"	11	"
"	"	150	"	"	"	"	10	"
"	"	200	"	"	"	"	9	"
"	"	400	"	"	"	"	6–9	"

There were some variations from the above figures, but, in the great majority of cases, the fruiting occurred with such regularity that the day of sporangium formation of a given plasmodial culture could be correctly predicted on the day it was started. The analyses of lights from the various types of bulbs used in the experiments show that variations in the length of time spent in the vegetative phase were due to the factor of intensity rather than to differences in quality of the various lights. Analyses of the types of bulbs used are shown in table 1.[2]

Table 1. *Analyses of electric light bulbs used in light intensity studies.*

Lamp size (watts)	Approximate lumens	Percentage of total light			
		Violet and blue	Green	Yellow	Orange and red
60	760	2.25	40.25	27.5	30.0
100	1530	2.3	40.7	27.4	29.6
150	2535	1.9	41.0	27.0	30.1
200	3400	2.5	41.5	27.2	28.8

In discussing various features of the plasmodial behavior of *Didymium difforme* (Pers.) Duby, Skupienski (1928, p. 329) made the statement: ". . . . if one submits a well-expanded plasmodium to the action of light rays, coming from a well-defined source, for two minutes only, and if one repeats this short illumination every two hours, the fruiting of the plasmodium in question is surely accelerated." This suggested the possibility that fruiting might be induced more rapidly by alternate periods of light and darkness rather than by continuous exposure. Moist chamber cultures were exposed to light from a 100 watt bulb for eight out of each twenty-four hours and were found to require one to two days longer for fruiting than those cultures continuously exposed to the same light. Cultures which were exposed to intermittent illumination from a 60-watt bulb (obtained by means of a flashing device that gave periods of 2.5 seconds of light alternating with periods of 0.5 second of darkness), remained in the vegetative state two days longer than cultures exposed to continuous illumination from the same type of bulb. It would seem from these results that alternate periods of light and darkness enable plasmodia to fruit with a much smaller total amount of light than required by plas-

[2] From the manufacturer's published tables.

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modia when they are continuously exposed. The cultures exposed to light from a 100-watt bulb for eight hours per day required only one or two days longer for fruiting than cultures continuously exposed, whereas, if a direct mathematical relationship existed, theoretically they would require thirty-three days, since they were exposed to light only one-third of the time. What actually occurred was that cultures continuously exposed required a total of 264 hours exposure to light for fruiting, while cultures exposed eight hours per day required only 96 to 104 hours of exposure, although they did spend a longer time in the vegetative phase than cultures continuously exposed.

TABLE 2. *Light transmission analyses of colored plates.*

	Wave length (millimicrons)						
Plate used	400	450	500	550	600	650	700
Blue	68.00	45.45	6.46	2.56	0.57	0.52	24.46
Red	6.00	0.56	3.29	3.84	16.09	33.68	43.61
Yellow ..	4.00	2.27	10.52	21.79	33.33	35.78	39.36

Experiments relative to the effect of lights of various wave lengths (of the visible spectrum) were first conducted with moist chamber cultures which were continuously exposed to lights transmitted through colored glass plates. One series was conducted, and then standardized gelatin filters were substituted for the glass plates. In the original experiment three colored plates were used: red, blue, and yellow. These plates were analyzed by means of a Razek-Mulder Color Analyzer; results of the light transmission analyses are summarized in table 2. From this table it may be seen that the blue plate passed a comparatively high percentage of light in the violet and blue bands of the spectrum, very little in the green and yellow, practically none in the orange, but passed considerable light in the red and near infra red. The red plate passed very little in the violet and blue bands, from 3.9 to 16 per cent in the green and yellow, increasing in the orange and red. The light transmission of the yellow plate was roughly similar to that of the red plate; however, it transmitted a greater percentage of light in the green, yellow, and orange bands.

TABLE 3. *Summary of results of one experiment using light transmitted through variously colored plates.*

Culture number	Color of plate used	Hours per day exposed	Length of vegetative phase
1	Blue	24	15 days
2	"	8	14 "
3	Red	24	Died
4	"	8	16 days
5	Yellow	24	Died
6	"	8	14 days

Six cultures were used in the preliminary experiment, the results of which are given in table 3. This experiment showed that the shorter wave lengths of the visible spectrum stimulate fruiting, while the longer wave lengths either retard or prevent fruiting. In later experiments in which Wratten Filters were used, only one, whose transmission analysis is shown in figure 1, induced fruiting. This would tend to support the view that the shorter wave lengths are necessary for fruiting. On the other hand, in the longer wave lengths of the visible spectrum or in infra-red rays, the plasmodia never fruited under conditions of continuous exposure.

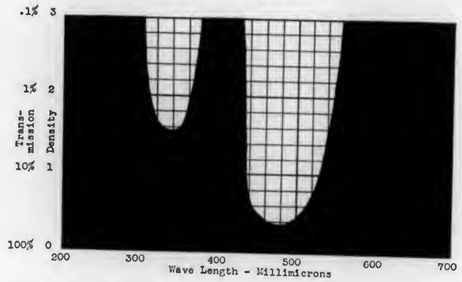


Fig. 1. Light transmission analysis of gelatin filter under which fed cultures of *P. polycephalum* formed fruiting bodies.

A number of investigators have maintained that the fruiting of myxomycetes is due solely to the depletion of nutrition. Camp (1937) has shown that plasmodia of *P. polycephalum* may be induced to fruit by starvation. The present study indicates that cultures which are not fed will spend less time in the vegetative phase than those which are fed, but this will scarcely explain why plasmodia will often fruit when they are supplied with abundant food materials. In order to determine whether or not light would have any effect on plasmodia to which no nutrient was supplied, a number of cultures were set up. As in previous experiments, five-day old stock cultures, which had been fed twenty-four hours previously, were used as sources of inocula. With few exceptions the light reactions were the same as those shown by fed cultures. A higher percentage of unfed cultures (inoculated from stock plasmodia maintained in the dark) formed sporangia in the complete absence of light than did fed cultures of the same type. Results of light intensity studies conducted with cultures of this type are summarized in table 4.

TABLE 4. *The effect of light intensity on fruiting in unfed cultures of Physarum polycephalum.*

Lamp (watts)	Number of cultures	Average length of time required for fruiting
60 W.	8	87 hours
100 W.	6	81 "
150 W.	21	78 "
400 W.	21	68 "

Experiments concerned with exposing unfed cultures to various wave lengths of light did not produce results which were in agreement with the results obtained when fed cultures were used. Sporadic fruiting occurred in such cultures, regardless of the light used, so the inference is that the influence of starvation is much stronger than any possible inhibitory effect of light in the longer wave bands.

Physarum tenerum Rex.—This species has the same type of bright yellow plasmodium as *P. polycephalum*, but as yet has not been induced to form large masses of protoplasm. Like *P. polycephalum*, plasmodia of *P. tenerum* grown in the light were a paler shade of yellow than those grown in the dark. A clumping of plasmodia occurred in the light but to a lesser degree than in *P. polycephalum*.

Numerous plasmodial cultures were exposed to direct sunlight in the greenhouse, and after forty-eight hours all had lost their color. When these cultures were placed in the dark, they failed to revive, thus showing that plasmodia of *P. tenerum*, like those of *P. polycephalum*, are killed by direct sunlight. In no case did plasmodial cultures fruit in the complete absence of light; over two hundred cultures were kept in the dark, and all remained in the active vegetative state as long as the nutrient agar remained moist. Many plasmodia remained active for as long as sixty days, and one persisted for seventy-six. Due to the fact that it needs transplanting so infrequently, this species is excellently adapted for laboratory cultivation, although, as mentioned above, it does not produce such large quantities of protoplasm as *P. polycephalum*. Preliminary experiments indicate that it can probably be adapted to moist chamber culture, using pulverized rolled oats as nutrient.

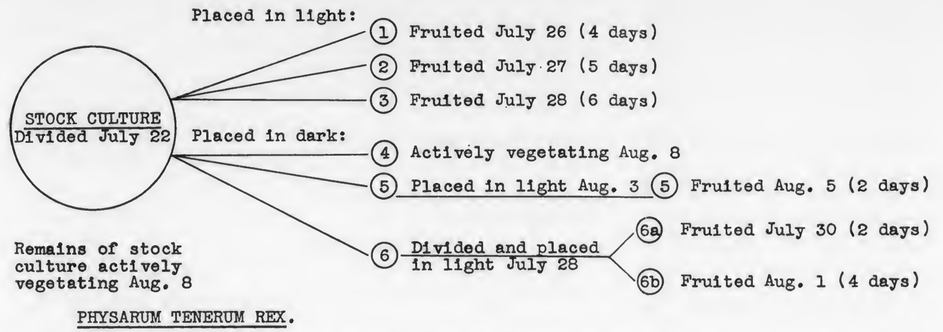
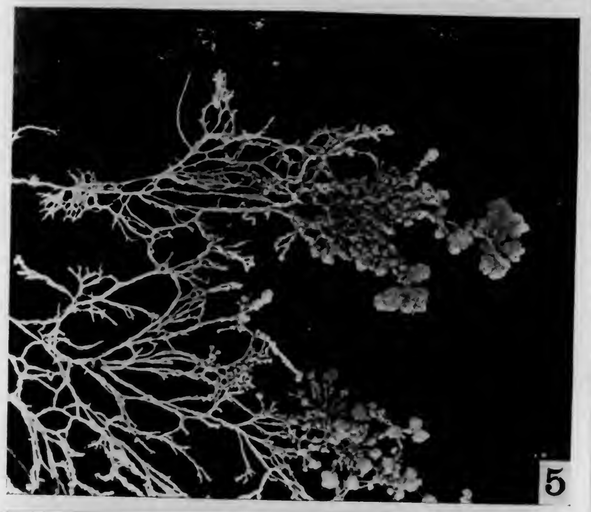
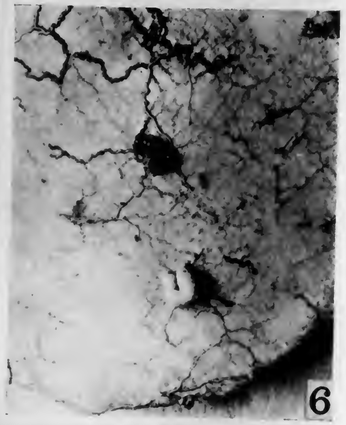


Fig. 2. Diagrammatic representation of the effect of diffuse natural light upon the fruiting of *P. tenerum*.

In diffuse natural light in the laboratory, different lengths of time were required for the formation of fruiting bodies by various plasmodial cultures. The results of one such experiment are shown in figure 2. This figure shows that the vegetative phase of *P. tenerum* varied from 2 to 6 days in natural light and well illustrates the necessity for more controlled conditions than are normally found in the laboratory. Seifriz and Russell (1936) apparently encountered similar difficulties, since they found that under laboratory conditions the rhythm of *P. polycephalum* varied from 9 to 23 days—a situation non-existent for this species (*polycephalum*) under conditions of constant light, temperature, and food supply. Under such controlled conditions some variation did occur in cultures of *P. tenerum*, but this variation was not nearly so great as that exhibited by cultures grown in natural light. The average length of the vegetative phase of twenty-four cultures grown under a 100-watt light was eighteen days, while under a 400-watt light the average length of the vegetative phase was thirteen days (15 cultures used). This difference is sufficient to show that the same relation exists between length of vegetative phase and light intensity as was exhibited by *P. polycephalum*. Plasmodia of *P. tenerum* possess a vegetative phase of somewhat longer duration than plasmodia of *P. polycephalum*.

Fuligo septica (L.) Weber.—Spores of this species germinate readily, and small, yellow plasmodia have frequently been obtained from spore sowings on corn decoction agar. These plasmodia, which failed to attain the size of those found in nature, grew very slowly, and none was ever observed to complete its life cycle. Attempts to maintain this species in moist chamber culture for any length of time have also failed; hence, all observations on this species have necessarily been made on plasmodia collected in the field. From these observations alone, it would seem that *F. septica* must have light in order to complete its life cycle, because young plasmodia, which were collected in more or less shaded habitats and kept in darkness, have never been observed to form fruiting bodies.





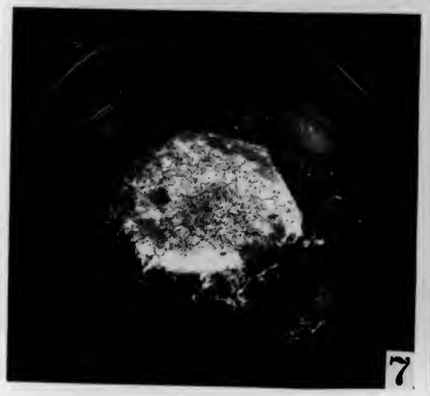


Fig. 3–9.—Fig. 3. Immature sporangia of *H. clavata*. × 6.—Fig. 4. Portion of plasmodium of *P. polycephalum*, grown in darkness. Natural size.—Fig. 5. Portion of plasmodium of *P. polycephalum*, grown in light. Natural size.—Fig. 6. Plasmodium of *F. septica*, showing clumping of protoplasm. × 1½.—Fig. 7. Moist chamber culture of *P. polycephalum*. × ⅓.—Fig. 8. Immature sporangia of *T. persimilis*. × 2.—Fig. 9. Beaker culture of *P. polycephalum*. × ¼.

Plasmodia of *F. septica* show the phenomenon of clumping in a remarkable degree when exposed to light. For this study, pieces of wood, on which active plasmodia were spread, were placed in moist chamber culture, and in a few hours most of the plasmodia had moved to the moist paper on which the pieces of wood were placed. When such plasmodia were exposed to light from a 100-watt bulb, at a distance of eighteen inches, a noticeable congregation of protoplasm into the larger veins of the plasmodial network became apparent at the end of one minute. At various points along these veins, swellings began to appear, and four to five minutes later some of them had grown as large as 1 cm. in diameter (fig. 6). Baranetzki (1876) mentioned the clumping of plasmodia of *F. septica* exposed to light but failed to mention the production of large plasmodial swellings such as those just described. Plasmodial cultures of *F. septica* on corn decoction agar were killed within twenty-four hours when exposed to direct sunlight.

Leocarpus fragilis (Dicks.) Rost.—In so far as a source of small plasmodia is concerned, this species is an excellent yellow-pigmented type for laboratory cultivation. Spores germinate readily, and plasmodia can generally be obtained in ten days or less from spore sowings on either corn decoction agar or hay infusion agar. These plasmodia need transplanting infrequently (every 2 or 3 weeks) and hence are easily maintained. No fruiting has been obtained as yet, and the suggestion is made that neither of the two media used is adapted for its cultivation. Like the yellow-pigmented types previously discussed, the plasmodia of *L. fragilis* clump and are bleached when exposed to light and are killed when exposed to direct sunlight.

NON-PIGMENTED PLASMODIAL TYPES.—In this group are placed those species in which the plasmodia are typically watery- or pearly-white. All species in the group herein discussed change color before the fruiting bodies mature, but the change occurs after sporangial delimitation has begun.

Physarum compressum Alb. & Schw.—The pearly-white plasmodium of this species was obtained easily from spore sowings on corn decoction agar. Plasmodia formed typical sporangia in the absence of light about six days after they were large enough to be visible without the aid of a lens. Attempts to maintain the species in culture by means of plasmodial transplants were, on the whole, unsuccessful. Disturbing the plasmodia markedly inhibits growth

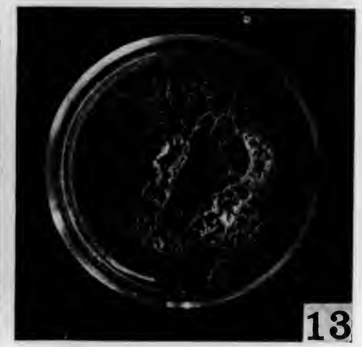
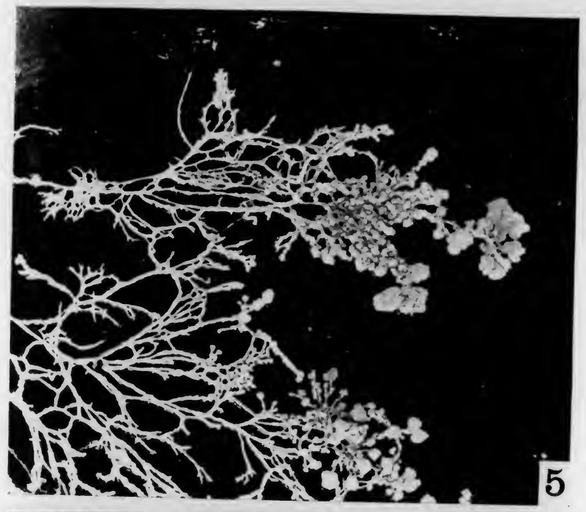
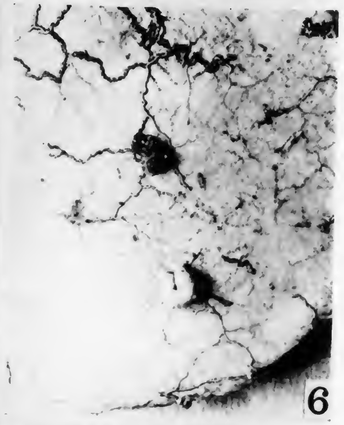


Fig. 10–13.—Fig. 10. Plasmodium of *S. fusca*. × 3.—Fig. 11. Same as fig. 10—6½ hours later. × 3.—Fig. 12. Same as fig. 10—4½ hours later. × 3.—Fig. 13. Petri dish culture of *D. xanthopus* on carrot agar. Natural size.





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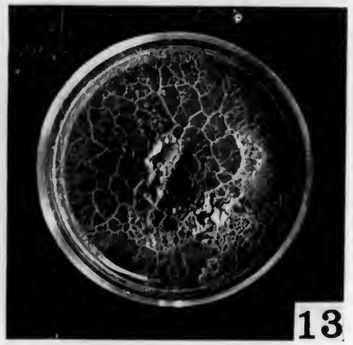
Non-pigmented plasmodial types.—In this group are placed those species in which the plasmodia are typically watery- or pearly-white. All species in the group herein discussed change color before the fruiting bodies mature, but the change occurs after sporangial delimitation has begun.

Physarum compressum Alb. & Schw.—The pearly-white plasmodium of this species was obtained easily from spore sowings on corn decoction agar. Plasmodia formed typical sporangia in the absence of light about six days after they were large enough to be visible without the aid of a lens. Attempts to maintain the species in culture by means of plasmodial transplants were, on the whole, unsuccessful. Disturbing the plasmodia markedly inhibits growth



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INTENTIONAL SECOND EXPOSURE

and fruiting, and cultures made by means of plasmodial transplants nearly always died within twenty-four hours, although in a few cases the life cycle was completed. Either the plasmodium of *P. compressum* is exceptionally delicate or else corn decoction agar is not the most suitable medium for its cultivation. However, attempts to grow this species on various other types of media yielded no better results.

Physarum nutans Pers.—Only one observation was made on the light relationships of this species. A small, white, net-like plasmodium, found under the bark of a decaying log of *Prunus serotina*, was transported to the laboratory, kept in complete darkness, and, after three days, formed typical sporangia of *P. nutans*. The plasmodium received no light except for a few moments when it was first discovered, so it is safe to infer that light is not prerequisite for the completion of its life cycle. Since the age of the plasmodium was unknown, no definite length of vegetative phase may be set.

Stemonitis spp.—The plasmodia of approximately half of the described species in the genus *Stemonitis* have not yet been observed, but in the majority of known cases they are white. This is true for both *Stemonitis axifera* (Bull.) Maebr. and *S. fusca* Roth (fig. 10, 11, 12), the plasmodial behavior of which has been described previously by the writer (Gray, 1936, 1937). All conclusions concerning these species are based on observations made on plasmodia collected in their natural habitats, as there are no records of either species having been artificially cultured, and to date no methods for their cultivation have been devised. From observations made by the writer, it is evident that light is not necessary for fruiting, since plasmodia which arose on decayed wood in moist chambers were able to complete their life cycles in the absence of light. Several observations have also been made on plasmodia which were collected under the bark of decaying logs and transported in darkness to the laboratory; fruiting of such plasmodia subsequently occurred in the complete absence of light. As in other species, in which the plasmodia inhabit the interstices of decaying wood, it will be difficult to establish a definite length of time for the vegetative phase: however, data at hand indicate that plasmodia of *S. fusca* remain in the vegetative stage somewhat longer than do plasmodia of *S. axifera*.

Comatricha typhoides (Bull.) Rost.—In a recent paper (Gray, 1937) the writer described the development of this species from white plasmodium to mature sporangia. The time required for completion of fruiting in the specimen described was twenty-three hours. Another such observation has been made, and the required time was fifteen hours. This variation in time was probably due to difference in ages of plasmodia, and since plasmodia of this species are similar in habit to those of various species of *Stemonitis*, it will be difficult to determine the normal length of time which they spend in the vegetative stage.

C. typhoides apparently does not need light in order to fruit, since plasmodia have frequently appeared and eventually formed sporangia on moist pieces of decayed wood kept in the dark.

Lamproderma arcyrionema Rost.—Spores of this species may be germinated easily in either tap- or distilled water, but as yet no plasmodial development has been obtained on any of the artificial media used. Plasmodia have been obtained, however, by sowing spores on small pieces of moist, decayed wood kept in the dark. Sporangia have been obtained in this fashion in the complete absence of light, but no data are available concerning the exact length of the vegetative phase. Like the previously described members of the order Stemonitales, *L. arcyrionema* possesses plasmodia which inhabit the interior of decaying wood; hence the age of plasmodia cannot be accurately determined. Since in several instances small, pearly-white plasmodia of this species arose on moist wood and formed sporangia in 12–14 hours, we may infer that *L. arcyrionema* possesses a relatively short rhythm in so far as the vegetative stage is concerned.

Hemitrichia vesparium (Batsch) Maebr.—The plasmodium of this species is listed in most taxonomic works as being deep red or purple red, but the writer has never observed anything but white plasmodia during the active vegetative phase. It is true that the color becomes red before the fruiting bodies are mature, but this color change occurs after sporangial delimitation has begun and not while plasmodia are still vegetating. It is recognized that plasmodial colors may be somewhat variable, depending upon the color of the substratum, but generally a radical change in plasmodial color during the vegetative phase is due simply to the engulfing of different colored particles of the substratum. Naturally, if a plasmodium should engulf red particles it would, for a time, appear red. This type of change in plasmodial color is exemplified by the photograph of a plasmodium of *F. septica* (fig. 6); at the top the color is brownish-yellow due to the engulfing of many, small, brown fragments of decayed wood on which it was growing before it was allowed to spread to the paper in the moist chamber.

H. vesparium needs no light in order to form sporangia. Numerous instances have occurred in which plasmodia have come to the surfaces of pieces of decayed wood, which had been placed in the dark, and there formed sporangia. Sporangia of *H. vesparium* have also been found on the walls and ceilings of mushroom houses, where the only light was an occasional flashlight beam.

Spore sowings in tubes of corn decoction agar have yielded pearly-white plasmodia which grow rapidly and are easily maintained through plasmodial transplants. In this type of culture fruiting was very erratic, so no data concerning rhythm or effect of light intensity are available. Fruiting frequently occurred, but in no case was an entire plasmodium utilized in sporangial formation. In several cultures, one sporangium was produced by a small bit of plasmodium and then was engulfed and destroyed almost immediately by the remaining plasmodial mass. As

yet the factors inducing fruiting in *H. vesparium* in this type of culture are unknown.

Hemitrichia spp.—Except for the species just discussed, no other species of the genus *Hemitrichia* were grown in the laboratory. However, several naturally occurring plasmodia of *H. clavata* (Pers.) Rost. (fig. 3) and *H. stipitata* (Massee) Maebr. were observed to form sporangia in the complete absence of light. Both species have been found in mushroom houses where they had fruited in darkness. The writer has observed only white plasmodia of either species, although Lister (1925, p. 221) states that the plasmodium of *H. clavata* may be either watery-white or rose-red. Sporangia of both species have been observed to pass through a rose-red stage, but this change of color occurs after sporangial delimitation has started.

made on twenty-two plasmodia of this species which fruited in the dark.

VARIABLE PLASMODIAL TYPES.—One species, *Didymium xanthopus* (Ditm.) Fr., has been placed in this separate group because of the variability of its plasmodial color. In the discussions of various yellow-pigmented plasmodial types it was noted that the bright yellow color fades to a certain extent when plasmodia are placed in the light. Plasmodia of *D. xanthopus* did not exhibit this type of variability, as any given plasmodium might vary from white, through various shades of creamy- or greenish-yellow to bright yellow, independently of light conditions. For instance, two plasmodial transplants might be made from a single yellow (or white) plasmodium, and the plasmodium of one of the resultant cultures would be white or cream-colored, whereas the other would

TABLE 5. *Summation of observations on twenty-two plasmodia of T. persimilis which completed their life cycles in complete absence of light.*

No. of plasmodia	Source of plasmodia	Time of collection	Fruiting completed	Hours required
1	Under bark of decaying cottonwood log	2:00 P.M. Sept. 8	8:00 A.M. Sept. 9	18
3	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:30 A.M. Sept. 14	17
1	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:00 A.M. Sept. 15	31
3	Appeared on moist wood in dark chamber	10:00 A.M. Sept. 14	8:00 A.M. Sept. 15	22
7	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	11:00 P.M. Sept. 15	9.5
3	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	8:00 A.M. Sept. 16	18.5
4	Under bark of decaying wild cherry log	2:00 P.M. Sept. 17	8:00 A.M. Sept. 18	18

Trichia persimilis Karst.—The plasmodium of this species is typically watery-white and does not change color until the separate sporangia have taken form (fig. 8). After the sporangia have assumed the shape and size of mature fruiting bodies, they become pale lemon-yellow and gradually darken until the shining golden- or yellow-ochraceous color of mature sporangia is attained. Attempts to culture this species in the laboratory on various types of media used for other species have failed, but numerous observations have been made on plasmodia collected in the field and brought to the laboratory. These plasmodia were all collected beneath the bark of decaying logs, where they received no light; they were then transported in darkness to the laboratory where most of them were placed in moist chambers in the dark. Fruiting bodies were formed both by sporangia kept in the dark and those kept in diffuse natural light. Since the age of each plasmodium is unknown, no definite rhythm may be set for *T. persimilis;* the indications are, however, that it has a comparatively short rhythm. Table 5 is a summation of the observations

be yellow. Plasmodia, regardless of color, formed sporangia equally well under conditions of artificial light or darkness, although there was no regularity in the duration of the vegetative phase of the various cultures—fruiting occurring any time between six and twenty-seven days. Plasmodial cultures were maintained in direct sunlight for periods as long as twenty days, but no cultures ever formed sporangia under such conditions.

It is evident from these results that light plays no important role in sporangia formation, and at present no theory is presented as an explanation of what causes fruiting in this species. There is a possibility that temperature is an important factor, since Skupienski (1934) maintains that different strains of this species have different optimum temperatures, although the race which he terms "*thermophilum*" (not implied in a systematic sense) showed very little variation in length of vegetative phase under temperature conditions varying from 6° to 25°C. If physiologic strains do exist, the interpretation of the results of other investigations, as well as the present

one, becomes a difficult task, since different strains may be involved in the various studies.

Discussion. — From the above observations, it is evident that under the conditions outlined yellow-pigmented plasmodia will not form fruiting bodies in the complete absence of light. This leads immediately to the conclusion that the pigment has an important function and is probably a part of the mechanism by which plasmodia are able to perceive the stimulus of light. The fundamental role of pigments in various living processes has been shown with many organisms, plant and animal, so this finding is not that of a new phenomenon but merely another example of a long-known one.

The chemical nature of the pigment has not been determined, but several theories have been advanced as to the nature of pigments that occur in myxomycetes. Seifriz and Zetzmann (1935) suggested that the yellow pigment of *Physarum polycephalum* belongs to the lyochrome or flavone group (of which the flavins are members). If the pigment is of the flavone type, a plant relationship would be suggested for the group, since such pigments as a rule do not occur in animal tissues. Solacolu (1932), working with pigments from the mature fruiting bodies of twenty-six species of myxomycetes, came to the conclusion that pigments occurring in these organisms are anthracenes. He also suggested a plant relationship, since anthracenes are found in some fungi and some higher plants, but generally not in animals, although phenanthrene, an isomer of anthracene, and its derivatives are known to occur in animals (Fieser, 1936). Whatever the chemical nature of these pigments may be, it is evident that light has a specific photochemical effect upon them, since plasmodia were observed to become paler when exposed to light. Stobbe (1908), working with various fulgides, has shown that these complex chemical substances not only change color when exposed to light but also that lights of various wave lengths cause different changes. For example, this worker found that triphenylfulgid became orange-yellow when exposed to light of 550 to 700 millimicrons but became dark brown at wave lengths of 440 to 550 millimicrons. The fact, as shown by Stobbe, that light of various wave lengths may induce specific photochemical effects may explain in part the behavior of plasmodia of *P. polycephalum* when exposed to the different wave bands. The work of Brandza (1926) would indicate that profound morphological changes may occur in myxomycetes as a result of the influence of light. This worker reports that plasmodia of some species can live in direct sunlight and that such plasmodia are extremely modified. For example, he states that *Physarum viride* (Bull.) Pers. and its variety *incanum* Lister are really forms of *P. nutans* Pers., which were derived from different colored plasmodia by exposure to direct sunlight. Such change in color indicates a definite photochemical effect.

It was noted that yellow-pigmented forms showed a tendency to clump when exposed to light, a finding in agreement with the results reported by other investigators. Baranetzki (1876), working with *Fuligo septica*, first mentioned clumping of protoplasm of plasmodia; this worker further stated that a slight increase in illumination causes a distinct retardation of streaming movements of plasmodia. Englemann (1879), working with the amoeba, *Pelomyxa palustris*, noted that when light is thrown upon a pseudopod of this organism, it is withdrawn suddenly. Mast (1911, p. 229) makes the general statment that light retards the activity of protoplasm and prevents the formation of pseudopods on the more highly illuminated side. Thus we can see that light causes a contraction of protoplasm of organisms other than myxomycetes, and, while it may simply be an orientation process with respect to the stimulus of light, it would appear to be more than a coincidence that plasmodia not only clump when exposed to light but also when about to form fruiting bodies, as Baranetzki has already pointed out.

Various workers (Baranetzki, 1876; Stahl, 1884, p. 167; Davenport, 1879) have shown that plasmodia of myxomycetes, which are known to react to light, are negatively phototropic. The anomaly presented by a negatively phototropic organism requiring light for one of its most important processes is evident. The fact that the above workers have demonstrated this negative phototropism does not necessarily mean that plasmodia will always react negatively, however, since it is known that certain organisms may change their tropic responses. For example, Keeble (1910, p. 41-42), working with two species of *Convoluta*, found that in bright light *C. roscoffensis* is positively and *C. paradoxa* negatively phototropic, while in dim light, *C. roscoffensis* is non-phototropic and *C. paradoxa* is positively phototropic. Loeb (1904) has shown that certain fresh-water crustaceans (*Gammarus, Cyclops, Daphnia*), which are naturally negatively phototropic, can be induced to reverse their tropic responses through the use of certain chemicals, particularly acids. It may well be that myxomycetes, like other organisms, have the power of changing their phototropic responses.

The fact that under controlled conditions of light, temperature, and nutrition, plasmodia fruited with great regularity would support the earlier view of Cayley (1929) that a certain periodicity (rhythm) exists in the production of spores. Among the Fucaceae, the periodical liberation of sexual cells has been described by several workers. Williams (1898, 1905), who noted a fortnightly liberation of sexual cells of *Dictyota dichotoma* off the coast of England and Wales, concluded that this periodicity was hereditary, but that in seas where there are tides, it might be influenced by the changes of amount of illumination. Hoyt (1907) described a monthly liberation of sexual cells of this same species at Beaufort, North Carolina, and Lewis (1910) found that it produced fortnightly crops at Naples and noted that the critical points in the life cycle (initiation of sexual cell rudiments and liberation) not only bore a relationship to the tides,

as shown by previous workers, but coincided exactly with the periods of maximum intensity of illumination. This latter worker hypothecated that *Dictyota*, in adapting itself to differing conditions at various localities, has acquired its habit of periodicity in response to different factors. Tahara (1909) described an identical periodicity in the fortnightly liberation of oogonia of *Sargassum enerve;* in a later work (1913), Tahara showed that the initial liberation in *S. enerve, S. Horneri,* and *Cystophyllum sisymbrioides* bore a relationship to the highest spring tide, but that the intervals between successive liberations varied, thus suggesting the possible influence of other environmental factors (illumination?). Keeble (1910, p. 33) found that light was a very important factor in the egg-laying of *Convoluta roscoffensis*. He found that alternation of six hours of exposure to light with eighteen hours of darkness was the optimum for egg-laying and noted that this was the same exposure received by *C. roscoffensis* during spring tidal periods at which its eggs are laid habitually.

In relation to the rhythmical fruiting of myxomycetes, Williams' view that periodicity might be hereditary is supported in part by the fact that certain species exhibit longer vegetative phases than others. For example, *Physarum tenerum*, under a 100-watt light, required eighteen days for fruiting, whereas, *P. polycephalum*, under identical conditions, required only eleven days. While rhythmical fruiting may possibly be hereditary to a certain extent, it is certainly not so innate as to be unalterable with respect to environmental factors. The writer has shown that in fed cultures of *P. polycephalum* fruiting rhythm may be changed by varying the intensity of illumination. Camp (1937) showed that by starvation of plasmodia the vegetative phase of this species could be markedly reduced. Experiments of the writer, involving both starvation and intensity of illumination, showed that both factors inter-reacted in influencing the time of fruiting. Differences in rhythms of species, then, tend to support the view that rhythmical fruiting is hereditary; yet experiments on yellow-pigmented plasmodia, involving lights of different intensities, show that this periodicity of fruiting may be modified.

SUMMARY

Our lack of knowledge of the biology of the myxomycetes is due to the fact that so few species have been cultured. The problem of immediate interest is the development of methods for culturing many species under laboratory conditions.

The effects of light upon the fruiting rhythms of four yellow-plasmodial types, ten non-pigmented plasmodial types, and one variable plasmodial type (*Didymium xanthopus*) were investigated. It was found that, with the species studied, yellow-pigmented types require light in order to complete their life cycles, while non-pigmented types and *D. xanthopus* fruit equally well in light or darkness.

Under controlled conditions of temperature, light, and nutrient, fruiting periods of pigmented types assumed great regularity. Under conditions of constant temperature and continuous illumination the lengths of the vegetative phase of *Physarum polycephalum* and *P. tenerum* are conditioned by the total amount of light received. Under conditions of intermittent illumination the vegetative phase of *P. polycephalum* may be lengthened, although the total amount of light necessary for a cycle to be completed may be greatly reduced. Fed cultures of *P. polycephalum*, when placed in lights of various wave lengths, formed sporangia only when exposed to the shorter wave lengths of the visible spectrum. The plasmodia of all yellow-pigmented types clumped and faded when they were exposed to light.

The laboratory cultivation of *Physarum compressum, P. tenerum,* and *Hemitrichia vesparium* is reported for the first time.

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A Note on Stemonitis fusca Roth

WILLIAM D. GRAY, University of Pennsylvania

The phenomenon of fairy-ring formation by certain fungi has long been known to mycologists, and numerous workers have reported and figured this type of sporophore arrangement among various agarics. Among the Myxomycetes, however, there have been few reports of fairy-ring formation. Macbride (1899, p. 35) reports such formations as being common to *Physarum cinereum* (Batsch) Pers., stating that the rings are small, measuring but a few inches in diameter. More recently, Poteat (1937) reports the finding of quite large fairy-rings of *P. cinereum*. This author states that he has found rings as large as fifteen feet in diameter. As far as can be determined from available literature, these are the only records of the production of fairy-ring formations by the fruiting bodies of Myxomycetes.

Of the several species of Stemonitis which occur in Indiana, perhaps the two most frequently encountered are *Stemonitis fusca* Roth and *Stemonitis axifera* (Bull.) Macbr. During the spring and summer months, except in exceptionally dry weather, sporangia of one or both of these species may be found in almost any shaded woodlot where moist, decaying twigs and logs occur. In the course of collecting trips throughout the southern part of the state, both species have been observed many times. Fairy-rings, formed by sporangia of *S. fusca*, have been noted

Fig. 1. Sporangia of *Stemonitis fusca* Roth showing two incomplete fairy-rings. Magnification x 2/3.

numerous times, but in no instance were sporangia of *S. axifera* nor any other species of Stemonitis found to be arranged in ring fashion. Such rings are quite small, never approaching the large size reported by Poteat for *P. cinereum;* the largest ring did not exceed three inches,

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Fig. 1. Sporangia of *Stemonitis fusca* Roth showing two incomplete fairy-rings. Magnification x 2/3.

numerous times, but in no instance were sporangia of *S. axifera* nor any other species of Stemonitis found to be arranged in ring fashion. Such rings are quite small, never approaching the large size reported by Poteat for *P. cinereum;* the largest ring did not exceed three inches,

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INTENTIONAL SECOND EXPOSURE
IRREGULAR PAGINATION

and the majority were about two inches in diameter. The explanation of such a ring formation is simply that the plasmodium arises at a certain point on the substratum, grows evenly, and creeps at an even rate from the point of origin. When the vegetative phase of the plasmodium is terminated and it enters into the fruiting phase (Seifriz and Russell, 1936), the plasmodium is in the form of a circle; naturally when sporangia are formed, they are arranged thus. In the case of *S. fusca,* fairy-rings were found only on uniformly decayed wood which possessed a fairly even surface. This is prerequisite for ring formation because any obstacle in the path of the advancing plasmodium, such as an uneven place in the surface of the substratum or an area where the texture of the substratum is different, leads to a change in growth rate of the plasmodium at that particular point and hence disrupts the circular arrangement; such rings are shown in Fig. 1.

The fact that *S. fusca* forms rings, whereas no other species of this genus has been observed to do so, leads to the conclusion that such a characteristic is probably a specific one.

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Observations on the Methods of Stipe-formation in Stemonitis and Comatricha

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During the course of the writer's observations on myxomycete plasmodia, it has become apparent that many generic as well as specific behavior differences are to be expected. By behavior is meant the various conformations and color changes that are passed through in the development of sporangia from plasmodia; the time required to complete fructification is also taken into account, although it is less likely to be a fixed character. Myxomycete fructifications[1] are of three types: aethalioid, plasmodiocarpous, and sporangiate; the last may be either sessile or stiped. Naturally the behavior of the plasmodium of a plasmodiocarpous species would differ from that of either a sporangiate or an aethalioid type. In addition to such differences, however, there occur differences between forms which possess the same general type of fructification; this is shown particularly well by members of the genera Stemonitis and Comatricha.

Stemonitis and Comatricha are closely-allied genera belonging to the family Stemonitaceae, and mature forms of members of the two genera, when examined macroscopically, show many resemblances—so many, in fact, that at one time the representatives of both genera were included in the same genus. Plasmodia of Stemonitis and Comatricha characteristically inhabit the interior of rotten wood, emerging only for fruiting; in the majority of known cases, the typical plasmodial color is white. A general feature in sporangia of both genera is the presence of a long central columella, which is simply an elongation of the stipe. Observations on species of both genera show that the process by which the stipe and columella are developed in Stemonitis differs somewhat from that process in Comatricha. It is hardly to be expected that stipe- and columella-formation of all the species of Comatricha would be uniform; this is also to be said for species of Stemonitis, since there are forms which may be considered more or less border-line species between the two genera.

A review of the literature yields but few references to the subject of stipe- and columella-formation. DeBary (1884) presented some observations concerning this process in *Stemonitis axifera* (Bull.) Macbr. (*S. ferruginea* of Ehrenberg), but his method of viewing the fruiting bodies was to harden them with alcohol and then render the protoplasm transparent with glycerine. By this method, according to deBary's figures (Figs. 2a and 2b), it may be shown that formation of the stipe and columella begins at an early stage in the development of the fruiting body. Bisby (1914), working on the development of the capillitium

[1]Macbride and Martin (1934) define these as follows: *aethalium:* a fructification in which all or a considerable part of a given plasmodium is involved and in which differentiation has not proceeded to the delimination of separate sporangia; *plasmodiocarp:* a fructification which has the interior structure of a sporangium, but which retains the netted form and outline of the plasmodium; this type is formed by the aggregation of protoplasm into a few of the larger veins of the plasmodium as it rests upon the surface of the substratum; *sporangium:* typically an erect fructification of definite form and structure for a given species, each sporangium representing only a small part of the protoplasm of a given plasmodium.

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in *Stemonitis fusca* Roth, included some material on the formation of the columella; his investigations, however, were largely cytological. Details of the development of *S. fusca*, from plasmodium to sporangia, were described by the writer (Gray, 1936); in this description no figures of the developing sporangia were presented. Jahn (1931) gave an account, accompanied by excellent photographs, of various stages in the development of *Comatricha nigra* (Pers.) Schroet.; he had previously published (1899) on this same species, incorrectly designated *C. obtusata* Preuss.

Were it possible to grow members of this family at will, the matter of observing the development of all species would be simplified; at present, however, one must rely upon finding plasmodia in their native haunts, removing them to the laboratory, and there observing them during the fruiting process. It has been the experience of the writer ordinarily to find emerging plasmodia of these two genera in the afternoon or evening; this is by no means, however, a set rule. The following summaries and the accompanying diagrams will serve, in a measure, to point out the main features of the stipe-forming and other developmental processes observed in the two genera. Figs. 1, 2, and 3 (with the exception of 2a and 2b) are diagrams of species observed by the writer; Figure 4 is redrawn from photographs by Jahn.

Stemonitis fusca Roth. (Fig. 1, *a-e*) Plasmodium pearly-white, relatively large.

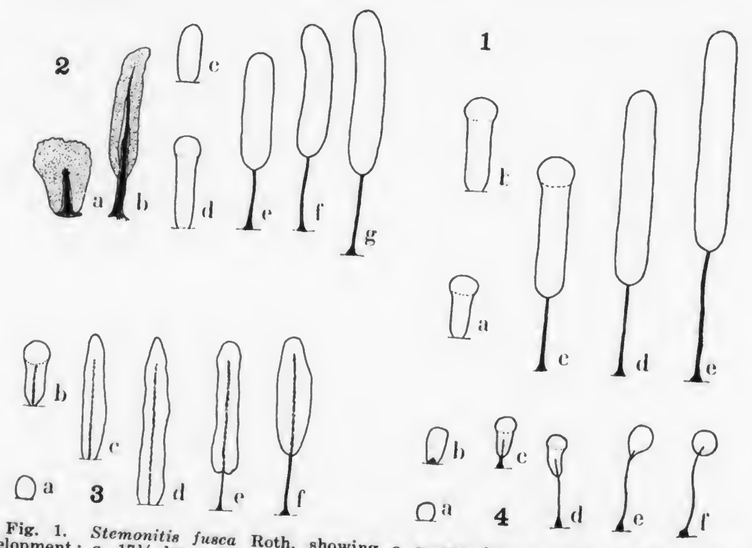

Fig. 1. *Stemonitis fusca* Roth, showing a sporangium in the various stages of development; *a*, 17½ hrs. after emergence; *b*, 19½ hrs.; *c*, 21½ hrs.; *d*, 26½ hrs.; *e*, 36½ hrs. Fig. 2. *Stemonitis axifera* (Bull.) Macbr. *a* and *b* (redrawn from deBary), longitudinal sections of young sporangia; *c-g*, a sporangium in the various stages of development; *c*, 3¼ hrs. after emergence; *d*, 8 hrs.; *e*, 12 hrs.; *f*, 17 hrs; *g*, 19 hrs. Fig. 3. *Comatricha typhoides* (Bull.) Rost. *a*, sporangium 8 hrs. after emergence; *b*, 10 hrs.; *c*, 11 hrs.; *d*, 13¼ hrs.; *e*, 13¾ hrs.; *f*, 14½ hrs. Fig. 4. *Comatricha nigra* (Pers.) Schroet. (redrawn from Jahn's photographs); *a*, sporangium shortly after emergence, the times listed for the following figures representing hours advanced past the stage shown in Fig. 4a; *b*, 2 hrs., 47 min.; *c*, 4 hrs., 43 min.; *d*, 7 hrs., 22 min.; *e*, 8 hrs., 55 min.; *f*, 9 hrs., 38 min. Magnification x4, except 2*a* and 2*b*, which are x6 and x7½, respectively.

Since the sporangia of this species occur in large clusters, the developing sporangia are intimately associated. Figure 1, a represents a single sporangium 17½ hours after the emergence of the plasmodium; at this point, the sporangium consists solely of a white, protoplasmic column, tipped with a bulbous enlargement. Figure 1, *b* is essentially the same as Figure 1, *a*, being a slightly more advanced stage. Figures 1, *c*, 1, *d*, and 1, *e* represent still further advanced stages in which the development of the stipe may be seen. The approximate time for completion of the fructification was 89 hours.

Stemonitis axifera (Bull.) Macbr. (Fig. 2, *a-g.*) Plasmodium pearly-white, developing into closely-associated sporangia as in *S. fusca.*

On the whole, plasmodia of this species are inclined to be more active upon emergence than are plasmodia of *S. fusca.* As may be seen from the diagrams (Fig. 2, *c-g*), development in the two species follows the same general lines, and, at certain stages, the species cannot be determined with certainty; however, there is a time variance, *S. axifera* requiring only 19 hours as compared to 89 hours required by *S. fusca.*[1] The two species pass through approximately the same color changes, white, pink, lavender, and finally brown, although the final shade of brown differs between the two species.

It is to be noted that in neither species of Stemonitis is there any external evidence of the existence of a columella. The sporangia are formed first, then the stipes are developed, appearing simply to lift the sporangia up from the substratum. Of course, the stipe is formed within the protoplasmic column, but obvious'y does not assume its final form and shining black color until it is exposed. Apparently this change in color takes place as the stipe is exposed. DeBary (1884) has shown that the formation of the stipe and columella begins in the very early stages in the development of *S. axifera;* this is not apparent to the observer, however, unless the immature sporangia are fixed and sectioned. Figures 2, *a* and 2, *b* are redrawn from deBary's figures of longitudinal sections of young sporangia of *S. axifera.*

Comatricha typhoides (Bull.) Rost. (Fig. 3, *a-f.*) Plasmodium white, but appearing somewhat finely granular and not of the same shining, pearly quality of whiteness exhibited by the two preceding species; rather active and inclined to divide into several smaller plasmodia, from some of which only one sporangium is developed; sporangia not closely aggregated.

Figure 3, a represents a sporangium in the very early stages (eight hours after the emergence of the plasmodium). Figure 3, *b* is the same sporangium two hours later; it is to be noted that a bulbous enlargement such as is shown by both species of Stemonitis is evident. The development of the stipe and columella has begun; they are dark in color, and their outlines show through the white protoplasmic mass. In Figure 3, *c* (11 hours after emergence), the protoplasmic column has elongated; dark stipe and columella are quite evident. This protoplasmic column continues to elongate until it has reached the maximum height of the fructification (Fig. 3, *d*), then the protoplasm contracts

[1]Further observations may show that the exact time requirements vary within a species.

upward along the central axis and thus exposes the already apparent stipe at the base of the fructification. Color changes are as follows: white, pale lavender, dark lavender, brownish-purple, and finally dark brown.

Comatricha nigra (Pers.) Schroet. (Fig. 4, *a-f.*) No observations on this species were made by the writer; diagrams drawn from Jahn's photographs have been introduced for the purpose of comparison. Figure 4, *a* represents a sporangium shortly after emergence, and Figure 4, *b*, the same sporangium two hours and forty-seven minutes later. At this stage may be seen the darkened central portion near the base, which marks the beginning of the formation of stipe and columella. Figure 4, *c* shows this sporangium about four hours and 43 minutes after emergence; the dark central portion, representing stipe and columella, has elongated; a portion of the stipe is already exposed. The stipe continues to elongate, and eventually the cylindrical protoplasmic column contracts along the stipe to form a more or less spherical sporangium about nine hours and 38 minutes after emergence (Fig. 4, *f*).

TABLE I. DEVELOPMENTAL FEATURES

SPECIES	Approximate Time Required	Color Changes in Order	Final Shape
S. fusca	89 hrs.	pearly-white pink pale lavender dark brown dark chocolate brown	cylindrical
S. axifera	19 hrs.	pearly-white pink pale lavender dark brown wood brown	cylindrical
C. typhoides	23 hrs.	granular white pale lavender dark lavender purple dark brown	cylindrical
C. nigra	?	watery white ? ? ferruginous or dark brown	spherical or oval

C. nigra differs from *C. typhoides* in that the stipe of the former continues to elongate after the basal portion is exposed. The early and obvious development of the stipe and columella is common to both species of Comatricha as is the contraction of the protoplasmic column along the central axis. On the contrary, at no time in the development of the two species of Stemonitis is the columella apparent. Known developmental features of the four species under discussion are summarized in Table I.

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A NEW DISTURBANCE OF RED PINE

RED or Norway pine (*Pinus resinosa* Ait.) has been regarded as a particularly suitable species for reforestation in the northeastern and lake states. The susceptibility of the more valuable white pine (*Pinus strobus* L.) to tip weevil and blister rust and the relative freedom of red pine from pests have caused the latter species to be given preference in many cases. The investigations now being carried on by the writer upon an unreported disturbance of red pine indicate that its freedom from disease is more apparent than real in many sections of the northeastern United States.

The external symptoms of the disease were first noticed by James A. Brock, assistant superintendent of the Rochester Municipal Watershed, in a plantation of young red pine in Ontario County, N. Y., during the summer of 1933. Since then the writer has found it or had reports of it throughout most of New York State, Connecticut and two counties in southeastern Pennsylvania. In gross aspect the symptoms resemble some types of insect injury, as the most conspicuous external characters are the extra-seasonal growth of one or more lateral buds in the terminal bud-cluster and the subsequent "forking" of the tree. The extra-seasonal growth of the lateral buds begins in June or July of the year that they are set, and may continue through August in the region of Hemlock Lake, N. Y. One or more buds are formed at the tip of these precocious shoots before growth ceases. The terminal bud of the parent shoot seldom takes part in this extra-seasonal growth and usually elongates at the normal time the following year. Since the abnormal or precocious laterals assume a more vertical position than is normal, the subsequent growth of the terminal bud causes a forked appearance of the tree. In some cases the original terminal may be forced to take the position of a lateral. This phenomenon, although undesirable, *per se* might be of no great consequence were it not for the fact that organic union of the wood usually fails to take place between the forked members during the later growth of the tree. Dissection of a tree, in which forking had occurred, ordinarily showed that the forked branch had failed to unite on its upper surface with the bole of the tree. A resinous pocket or fissure, surrounded by discolored wood, usually occurs at these areas of non-union. Such defects afford an ideal environment for many species of fungi, some of which are known to be parasitic.

Representative plots from approximately 800 acres of red pine, ranging in age from 5 to 25 years, have shown forking in 68 to 94 per cent. of the trees. The affected trees seldom, if ever, remain permanently forked, as the more vigorous member of the fork assumes a completely vertical position after a few years' growth and the other member tends to take the position characteristic of a normal branch. Hence the presence of the disturbance in a stand of red pine may not be evident to a casual observer until the invasion of parasitic fungi takes place.

Forking has been found on soils varying in pH from 4.5 to 7.5 and ranging in texture from sandy to clay loam. It has been observed in young natural reproduction as well as in plantations and in both mixed and pure stands. Investigations are being carried on in an effort to determine the primary cause and mechanism of forking. The evidence at hand strongly suggests a fungous origin.

JOHN AUSTIN JUMP

DEPARTMENT OF BOTANY,
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A NEW FUNGUS ON *FICUS NITIDA* THUNB

JOHN A. JUMP, *Graduate Student*,
University of Pennsylvania.

While visiting at the Agricultural Experiment Station in Río Piedras during the summer of 1936 the writer became interested in a fungus appearing upon *Ficus nitida* Thunb. which had been sent to the laboratory for identification. Subsequent study of the organism at the botanical laboratory of the University of Pennsylvania indicated that it was an unreported species of *Hypoxylon*. The name *Hypoxylon borinquensis* is proposed by the writer and the species is herewith described:

Hypoxylon borinquensis sp. nov. Stroma latissime effsa, carbonaceo, nitida, subsuperficiale in trunci ramorumque cortice, aliquid scabra propter ostiolis papillis, extus intusque nigrum. Peritheciis prerique, ovoides-oblongis, .2—.4 mm. lata. Ascis cylindraceis, densissime stipatis, brevissime stipatatis, aparaphysatibus, 130–160 × 7–8μ, octosporis. Sporidiis monostichis, subacutis, fusiformibus v. ovatis, saepe inequalateralbus, subflavis, 16–18 × 3.5–5μ. Hab. in ramis truncibusque Fici nitidi in Puerto Rico.

Stroma broadly effused, carbonaceous, shining, subsuperficial in bark of the trunk and branches; somewhat rough due to the papillate ostioles; black within and without. Perithecia numerous, avoid-oblong, .2—.4 mm. wide. Asci cylindrical, very densely crowded, very short stipitate, 130–160 × 7–8u, eight spored. Ascospores monostichous, subacute, fusiform or ovate, often inequilateral, yellowish, 16–18 × 3.5–5μ. Habitat in branches and trunks of Ficus nitida in Puerto Rico.

The stroma in gross form bears some resemblance to both *Eutypa erumpens* Massee and *Nummularia tinctor* Berk. and, since these fungi are also found in many of the islands of the Wset Indies, a careful examination should be made of diseased Ficus in order that the organism involved may be correctly determined. *Eutypa erumpens* has been reported as occurring on *Ficus nitida* and Cacao in Trinidad.

Is is quite difficult in many cases to identify the genus of a fungus in the group of the *Spheriales* which includes *Eutypa*, *Diatrype*, *Hypoxylon* and *Nummularia*, as they are similar in many respects

and have transitional forms of which the generic status is debatable *Hypoxylon borinquensis* corresponds in certain respects to *Nummularia tinctor* and perhaps should be regarded as a *Nummularia*, but the writer is included to consider *Nummularia* an invalid genus. Ellis & Everhart (1) refer to the genus as being too close to *Hypoxylon* in many of its forms and Miller (2), points out that the conidial layer actually arises in the same manner in both genera, although it was upon this character that the two genera were originally separated. *Nummularia* supposedly possesses a substromal conidial layer which is not found in *Hypoxylon*, but Miller has proved this distinction to be fallacious and concludes: "It is only logical to place the species that were taken out of *Hypoxylon* and placed in *Nummularia* back in *Hypoxylon*." The writer concurs with this point of view and accordingly has placed the fungus from Ficus in the genus *Hypoxylon*.

Hypoxylon borinquensis forms an unusually large stroma on its host. The average dimentions were not included in the description, as there was not enough material available to make a significant average, but a single stromatic surface of 1 dm. in width by 1–3 dm. in length might be expected when occurring on a limb or trunk of a sufficiently large diameter.

Botanical Laboratory
University of Pennsylvania
Philadelphia, Pa.

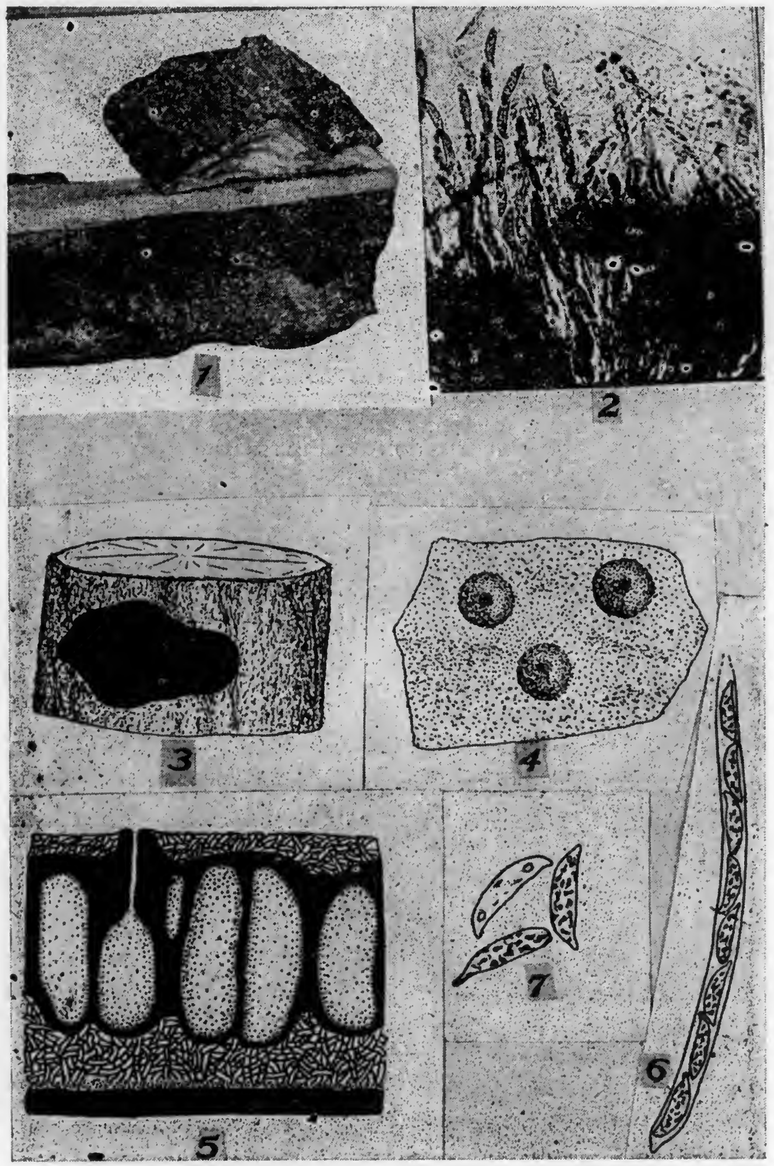
1. Collens, A. E. Dept. of Agri. of Trinidad. (n.s.) **61**: 33–43, 1909.
2. Ellis & Everhart North American *Pyrenomycetes*. Newfield 1892.
3. Miller, J. M. Biologic Studies in the Spheriales.
Mycologia **20**: 1928.

Explanation of Plates

Fig. 1.—Stroma on wood of *Ficus nitida*.
Fig. 2.—Ascospores of *Hypoxylon borinquensis* sp. n.
Fig. 3.—Habitat drawing.
Fig. 4.—Surface of stroma showing ostioles. Enlarged.
Fig. 5.—Diagrammatic section through stroma. Asci omitted.
Fig. 6.—Asceus of *H. borinquensis*
Fig. 7.—Ascospores.

IRREGULAR PAGINATION

PLATE XIX



A STUDY OF FORKING IN RED PINE

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE
UNIVERSITY OF PENNSYLVANIA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
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Reprint from
Phytopathology, Vol. 28, No. 11

PHILADELPHIA
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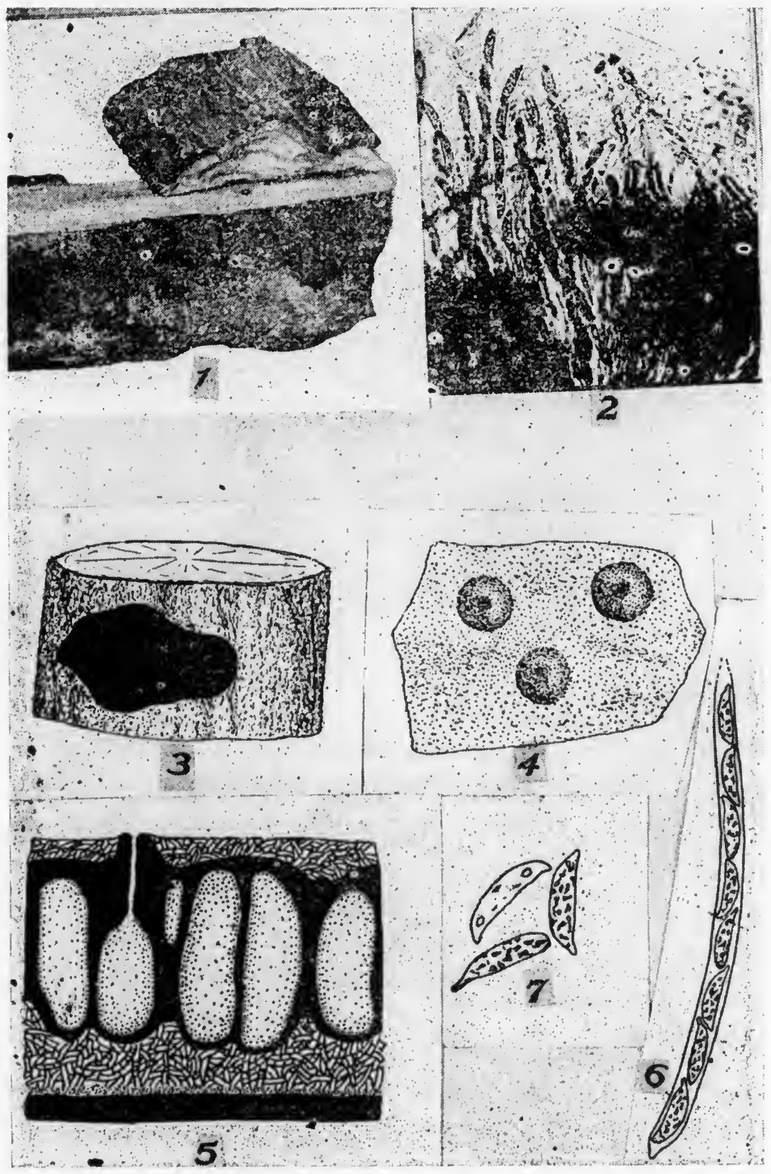

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INTENTIONAL SECOND EXPOSURE

[Reprinted from PHYTOPATHOLOGY, November, 1938, Vol. XXVIII, No. 11, pp. 798–811.]

A STUDY OF FORKING IN RED PINE[1, 2]

JOHN AUSTIN JUMP[3]

(Accepted for publication July 12, 1938)

INTRODUCTION

A high percentage of false bifurcation of the current season's growth of red pine, *Pinus resinosa* Ait., was noted in a forest planting on the eastern side of Canadice Lake, N. Y., by J. A. Brock in the spring of 1933 (9). The writer found the same disturbance in 3 counties in western New York State, in the northern Adirondacks, and in 2 counties in southeastern Pennsylvania in the summers of 1936 and 1937, although no systematic attempt was made to scout for it. H. H. York has reported it as occurring in plantings in Connecticut and several counties in central and eastern New York.

Unless specifically designed otherwise, all data included in this paper were obtained from plantings on the Rochester Municipal Watershed in Livingston and Ontario Counties, N. Y. Trees in these plantings varied from 5 to 25 years of age.

Kienholz (10) described a type of fasciation that might be considered as a tendency of the species to susceptibility to morphogenic disturbances, but there seem to be no references in the literature to the phenomenon of forking. Witches'-brooms do not appear to have been reported as occurring on red pine, although the writer observed one near the top of a tree in a 22-year-old planting.

SYMPTOMS

The first apparent symptom of forking is the extra-seasonal growth of lateral buds in the winter-bud cluster of the terminal shoot of the tree (Fig. 1, B). Extra-seasonal growth becomes noticeable in late June or

[1] A dissertation presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to express appreciation to Prof. H. H. York for advice and criticism during the course of this investigation, and to Prof. J. R. Schramm and Prof. Walter Steckbeck for criticism of the manuscript. Grateful acknowledgement is made to Mr. J. A. Brock for cooperation in the field investigations.

[3] The greater part of this investigation was carried on during the tenure of a George Leib Harrison Fellowship at the University of Pennsylvania.

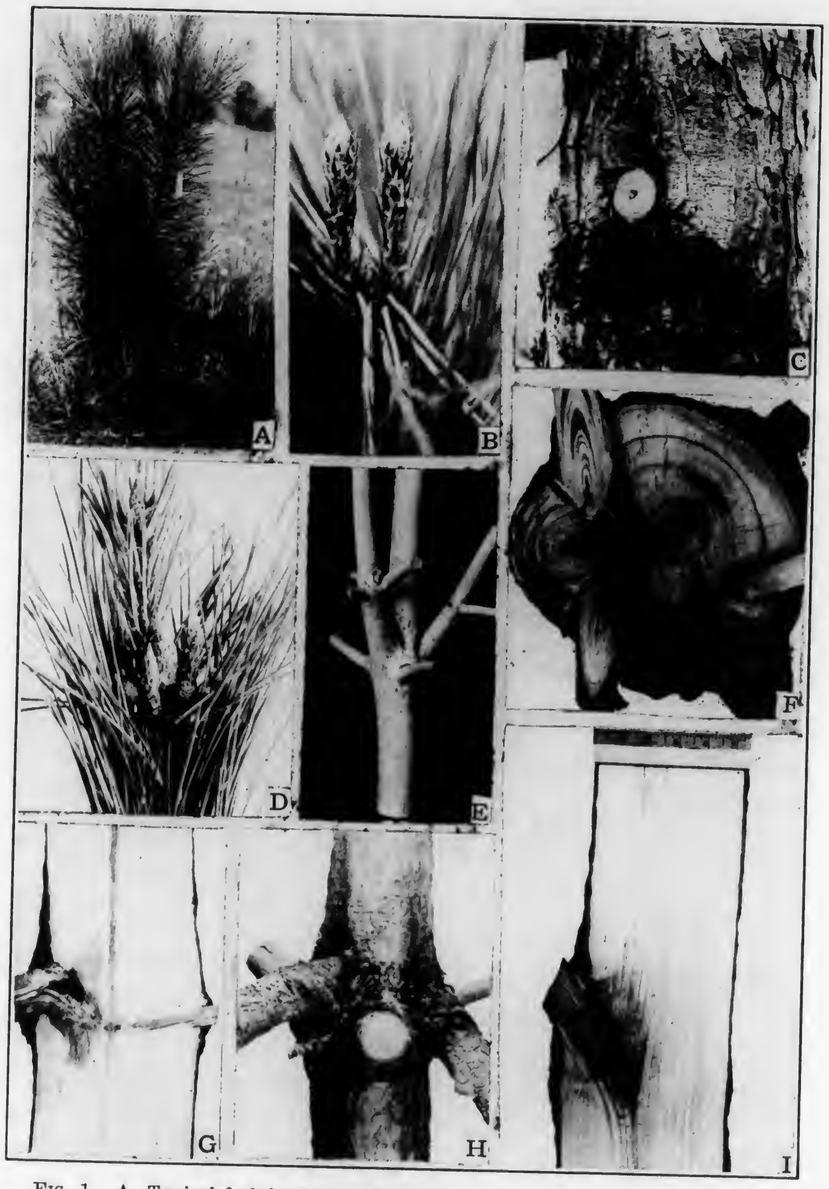


FIG. 1. A. Typical fork in 7-year-old red pine. B. Extraseasonal growth of lateral buds taken July 5 near Springwater, N. Y. C. Pruned forked branch on a 22-year-old tree with depression immediately above. D. Precocious shoot rising from bud cluster with short needles and two buds at apex. E. Peeled fork showing secondary branching. F. Cross section near a fork upon which secondary branching had occurred. G. Radial section of trunk showing effect of ice breakage upon a forked branch. H. Effect of weight of ice upon two forked branches near top of 22-year-old tree. I. Radial section showing characteristic appearance of a fork, with reddish-brown stain extending into the wood.

July, when 1 or 2, and in some cases as many as 4 lateral buds commence to grow. The resulting precocious shoots may attain a length of 3 to 4 inches, with 1 or more winter buds at their apices, before becoming dormant (Fig. 1, D). Needles develop on such shoots, but seldom attain more than half of their normal length and fail to elongate further at the initiation of growth the following spring. The precocious shoots may continue to elongate until quite late in the current season, although, according to Gustafson (5), elongation in pine shoots has been completed by early July in Michigan. Measurements of 10 precocious shoots in a planting of 8-year-old red pine were taken on August 15, 1937, and again on October 25. All but one of them showed an increase in length from $\frac{1}{4}$ to $1\frac{3}{4}$ in. The formation of a bud cluter at the tip of the precocious shoot (Fig. 1, D) interfered with normal subsequent growth, as it caused secondary branches to be formed within a few inches of the bole (Fig. 1, E).

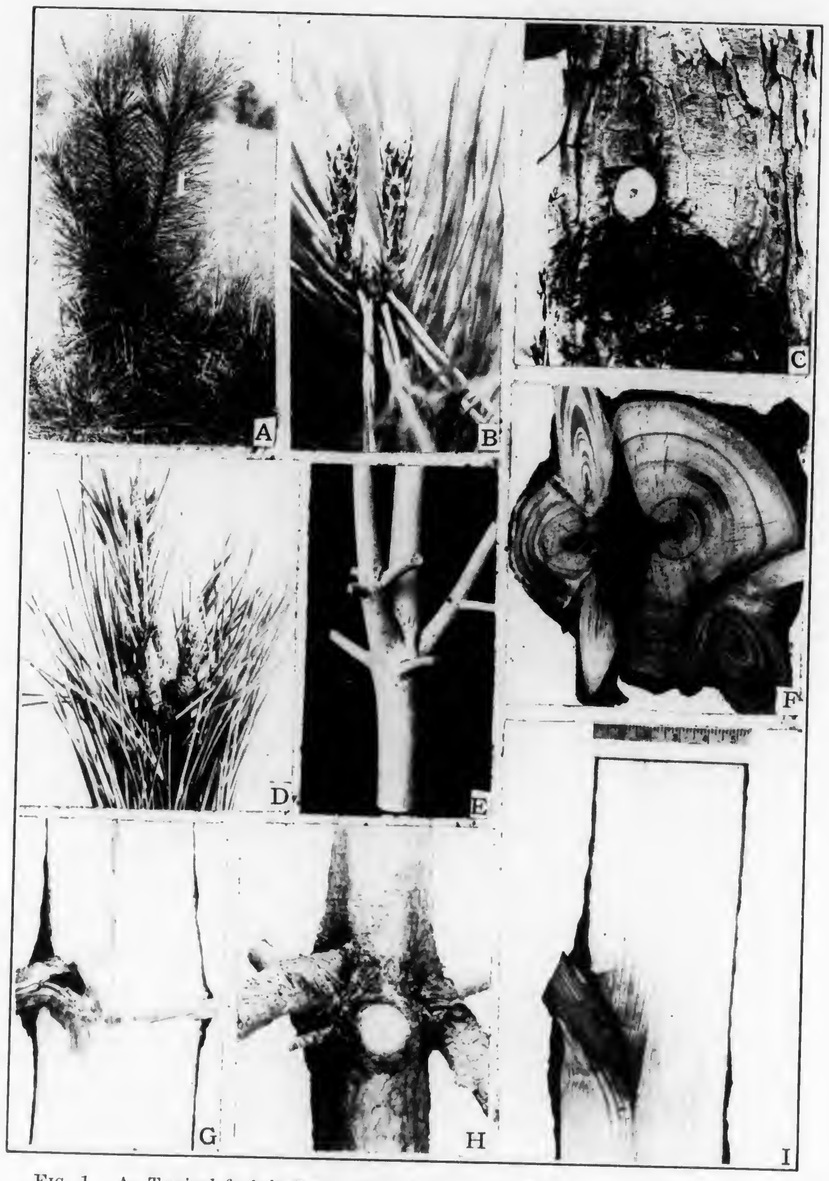
The terminal bud of the cluster in which the extra-seasonal growth occurs seldom takes part in the abnormal development, and usually elongates at the normal time the following season. The laterals, however, during the course of their extra-seasonal growth, react as if the terminal bud had been injured or removed and grow in a more or less vertical direction. Thus the shoot, originating from the terminal bud the following season, grows in competition with one or more laterals that had commenced growth the preceding summer. This results in a bi- or trifurcated appearance of the tree, which will be referred to as "forking" (Fig. 1, A). This condition does not remain conspicuous for more than 2 or 3 seasons in most cases. Either the true leader or one of the precocious laterals will assume dominance and the other member, or members, of the fork will tend to the more or less horizontal position normally characteristic of red pine branches.

Forking may occur repeatedly in a tree. One 22-year-old specimen, when dissected, showed this phenomenon in at least 12 nodes (Fig. 2). An examination of nodes where forking had occurred at some previous time frequently showed a slight depression in the trunk immediately above the insertion of the branch (Fig. 1, C).

There seems to be no relation between the age of a tree and its susceptibility to forking, in stands up to 25 years of age. No data have been obtained upon older trees because of the difficulty of making certain, without felling the tree, that a fork might have been induced by insect or mechanical injury.

DAMAGE RESULTING FROM FORKING

The structural injury that results from forking usually has its inception during the season following the formation of the fork, and is caused by the failure of the forked members to completely unite as they increase in diameter. Because of the acute angle at which they stand in relation to each other, they are unable to slough off, or push aside, the bark that lies on the inside of the fork between their cambiums, in the manner that the growth of the trunk displaces the bark of a normal lateral branch. This



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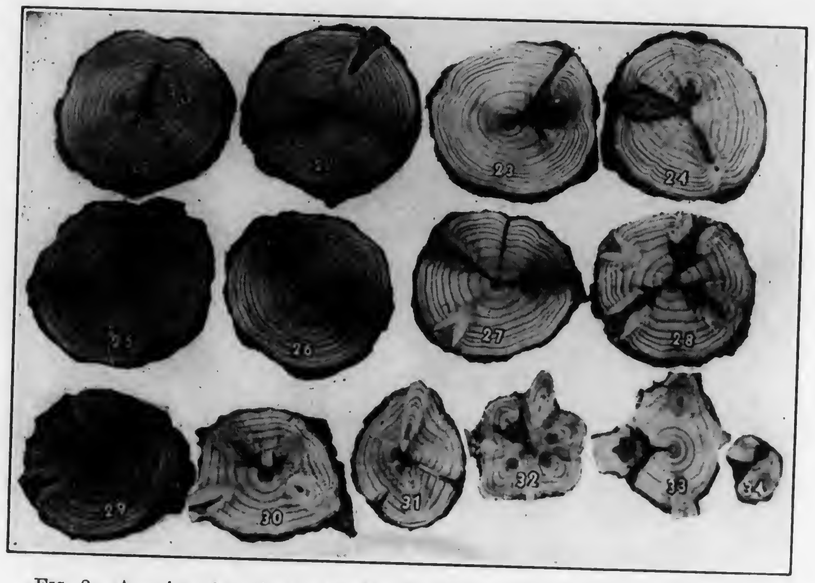


FIG. 2. A series of sections taken from a 23-year-old red pine in consecutive years from 1921 to 1934 inclusive, to show occurrence of forking and discoloration surrounding the pith.

area of non-union of cambium remains in the main axis of the tree as a fissure or pocket that becomes filled with a heterogeneous material composed largely of resin and fragments of the bark (Figs. 1, F and 1, I). These fissures are in a more or less vertical plane for several years until one of the forked members assumes dominance, as described above. While in this vertical position they are easily penetrated by water, and a favorable environment is thus established for the development of many species of parasitic and saprophytic bacteria and fungi. The presence of this flora may be an additional factor in causing the fissure to remain open.

Although it is not within the province of this investigation to evaluate the damage caused by forking from the point of view of the defects in the timber of forked trees, mention should be made of the increase of winter damage in forked stands. Since part of a forked branch may not be united organically with the trunk, the mechanical resistance to stress caused by weight of snow or ice is reduced. A branch that breaks in such a manner (Fig. 1, G) produces a more serious injury to the tree than does the breaking of a sound branch, as it lays open a cavity that may extend almost to the pith (Fig. 1, H). Normal branches are able to stand much greater stress and, if breaking occurs, usually snap off at a point beyond the trunk.

FORKING IN RELATION TO ENVIRONMENT

There appears to be no marked correlation between forking and site conditions, except a possible relationship with the mean seasonal temperature

of the locality. Determinations of soil pH in plots of forking red pine showed a range from 4.5 to 7.5 without appreciable difference in the percentage of forked trees. The disturbance occurred on soils varying from sandy types in parts of the Adirondacks to the clayey loam upon which the pine had been planted in Montgomery County, Pennsylvania. The most prevalent soil types on the Rochester Watershed were Volusia loam and Volusia shale loam. Degree and aspect of slope likewise seemed to be of no significance. There was no relation between forking and extremes of precipitation, or years of abnormal temperatures. The lowest percentages of forking encountered were in natural stands and plantations of red pine near Mt. Discovery, Essex County, New York. This locality was the most northern and the highest in altitude of those studied, which suggested that temperature and latitude might be possible limiting factors of the disturbance. Numerous observations of comparable sites, however, are necessary before any positive statement can be made on this point. Obviously, a shorter growing season would tend to minimize the effects of extra-seasonal growth, even though it might have no effect upon the primary cause.

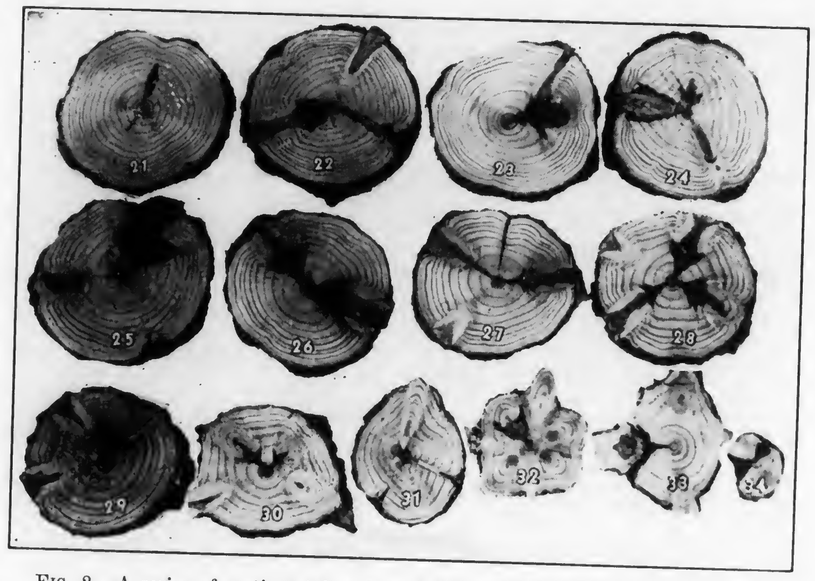
FUNGI FOUND ASSOCIATED WITH FORKING

Isolations

Cultures were made from red pine wood immediately after sawing. The surface of the wood was sterilized by swabbing with alcohol and flaming an area of several square inches in the vicinity of the place from which the sample was to be taken. A small piece of wood in the desired locality was then excised with a sterile woodcarving gouge, flamed briefly, and thrust into a tube of 2 per cent Fleischman's Diamalt Agar.

Cultures from buds were made by dipping the bud in 70 per cent alcohol and flaming it until the alcohol was burned off and the bud scales were partly charred. The bud was then split longitudinally with a sterile scalpel and the freshly cut surfaces were placed in contact with an agar slant.

Blue-green molds commonly occurred in cultures from discolored wood from the interior of a bole in the vicinity of a fork. The majority of these are believed to be inhabitants of the wood, and not the result of poor laboratory technic, for they usually did not appear for ten days or more following inoculation of the tube of medium with the wood sample. Bacteria were occasionally present, but no attempt was made to examine them in detail, although it was ascertained that no given form was consistently isolated. While only a limited number of trees above 20 years of age could be removed from the plantings for detailed examination, some indication of the internal condition of about 30 forked trees was obtained by means of an increment borer. Frequently, it was impossible to remove a complete core with the borer because of wood rot in the vicinity of old forks about waist high on the tree. Forked areas were almost invariably accompanied by reddish brown streaks within the wood (Figs. 1, I and 2) from which fungi could be isolated. These streaks often were found surrounding the pith in forked trees.



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INTENTIONAL SECOND EXPOSURE

A species of *Tympanis,* closely resembling *T. pinastri,* was cultured in several cases from discolored wood within the trunk adjacent to fissures caused by forking, and also from the interior of forked branches near their origin in the interior of the trunk. In one instance this species was cultured from the wood of a 7-year-old tree that was, apparently, in a perfect state of health, except for a fork that had occurred 2 years earlier.

Comparison of the species of *Tympanis* found in this investigation with cultures of *Tympanis* sp., kindly furnished by Dr. J. R. Hansbrough, indicated that the organism was the same species that was involved in his studies of the Tympanis canker of red pine (7, 8).

There is considerable evidence that *Tympanis* enters the tree, in the majority of cases, through fissures resulting from forking. The writer also believes that the depressed areas on the trunk above the insertion of a branch may, in many instances, result from the internal fissure caused by forking. As such depressions may, according to Hansbrough, be a symptom of *Tympanis,* a careful study of the internal anatomy, in addition to cultural investigations, seems advisable before making a diagnosis in the absence of fungus-fruiting bodies. Depressions accompanied forked branches in the majority of cases studied, in which the node was 10 or more years of age. Observations based upon the external appearance of depressions of 118 trees showed that the branch at the base of the depression had been a member of a fork in at least 84 per cent of the cases. The true figure probably would approach 100 per cent, since previous forking could not always be determined from external observations on account of the size and age of the nodes near the base of large trees and close pruning of lower branches in the stand.

Association of Dematium pullulans with Forking

The organism most consistently cultured from forked red pine was identified by Dr. David Linder of the Farlow Herbarium as a variety of *Dematium pullulans* de Bary.

Dematium pullulans was cultured from the lateral and terminal buds, pith, discolored wood surrounding the pith, and discolored wood in the root crown of red pine from the Rochester Watershed. It was also cultured from buds of forked red pine growing in southeastern Pennsylvania. It was the only organism to develop in significant percentages from red pine buds. Approximately 75 buds were used as material for culture, and 64 per cent of the resulting organisms proved to be *D. pullulans.* It also was found occasionally in cultures from other parts of the tree: twice in forked branch traces, twice in the pith, and once in the root crown. Cultures from the roots failed to show *D. pullulans,* although it is possible that it was not noticed because of the vigorous growth of bacteria and blue-green molds in some of the cultures. Although positive identification of the fungus in the tissues could not be made by means of an histological study, the presence of a catenate type of hyphae in the pith (Fig. 4, F) strongly indicated that the fungus under observation was *D. pullulans.* The probability of the

accuracy of this determination was strengthened by the lack of any cultural evidence of the presence of fungi possessing a mycelium of this type other than *D. pullulans.* Resistance of the hyphal wall to Cartwright's stain (1), which characterizes the chromatic hyphae of *D. pullulans,* was also typical of the pith-inhabiting organism.

Several members of the *Dematiaceae* have been recognized for some time as wood-inhabiting organisms. Siggers (13) refers to *Torula ligniperda* as a cause of structural weakness and brown staining in tulip poplar and white ash. Reddish-brown staining was usually present in the vicinity of old forks in red pine wood and was of frequent occurrence surrounding the pith in trees affected by the disturbance (Fig. 2).

Dematium pullulans is readily grown upon a wide variety of culture media, both liquid and solid. Cultures in flasks on sterile red pine sawdust, moistened with distilled water, produced a mycelium and conidia, but the yeasty conidial masses, characteristic of its growth on other media, were not evident. A series of cultures was made by placing a block of agar 5 mm. square, containing the fungus, upon the surface of steam-sterilized soil in large culture tubes. Growth took place readily and the hyphae penetrated the soil to the bottom of the tube: a depth of about 6 cm. Single-spore cultures were made by means of the Kienholz technic (11), and the following observations were made on sub-cultures of these on prune agar and on liquid 2 per cent malt.

MORPHOLOGICAL OBSERVATIONS UPON DEMATIUM PULLULANS

Dematium pullulans de Bary: The mature conidia are typically ovoid to ellipsoidal, averaging $7.4 \times 4.0\ \mu$, 2-guttulate and nearly hyaline. Variations from this type occasionally occur (Fig. 4, A), especially in liquid culture. In hanging-drop cultures the spores may reproduce by budding or germinate directly, forming hyphae upon which conidia may be borne laterally (Fig. 4, E). Freshly transferred cultures on prune agar produce conidia so abundantly that a yeasty mass, varying from white to light violet, is formed upon the surface of the culture. The vegetative mycelium is usually somewhat submerged and black in macroscopic appearance, forming a compact stroma-like layer at the surface. The mycelium varies from a dark *Torula*-like form, frequently found in liquid cultures, to a hyaline, distantly-septate type. It may contain granular material or drops of oil in some instances (Fig. 4, E). Round, black bodies, usually less than 1 mm. in diameter, often appear on the surface of prune-agar cultures 2 weeks or more old. These structures are composed of a black, highly-compacted outer layer with a pseudo-parenchymatous, readily stainable central core. Such bodies will be referred to as microsclerotia. Dark-ringed tapering hyphae often appear in tufts on the surface of microsclerotia, although they may also arise directly from the mycelial surface. Bulbilous structures, apparently of similar nature to the rings, frequently occur at the base of a hyphal branch. A type of exospore, resembling spores of the genus *Coniothecium,*

was found among these hyphae and was abundant also in liquid cultures. These spores were ovate to orbicular and usually very dark. They averaged 24 μ in length, and often had one or more hyphal cells attached to their distal end.

A graded pH series was set up with Elliott's medium (3), which contains asparagin as a nitrogen source; and a second series was run with 2 per cent malt. The agar was omitted from these media and the pH was adjusted with HCl and NaOH. The malt series of 12 pH values from 1.8 to 8.5 showed growth from 1.8 to 7.2, although growth was slow at the extremes of the range, particularly at the acid end. Optimum growth occurred between 5.5 and 6.5. On Elliott's medium at pH values from 2.2 to 8.4, growth was obtained from 2.2 to 7.2, with an optimum range betweeen 5.0 and 6.5. The malt series was inoculated with mycelium, while conidia were used with Elliott's medium.

Growth of different cultures of the fungus collected from various localities showed no tendency toward morphological variation when grown upon 2 per cent malt agar in Petri dishes. Numerous varieties of *Dematium pullulans* have been described, but the variety with which we are here concerned cannot be placed satisfactorily among these. In the character of its nodose hyphae it resembles var. *fimicola* Marchal, but differs markedly from it in spore characters.

EXTENT OF FORKING IN STANDS

A partial summary of data obtained from some of the plots studied on the Rochester Watershed is given in table 1. It is evident from data upon plots C and D that the more vigorous trees are the most susceptible to forking. This seemed to apply to trees in all of the age groups studied, although quantitative data are not available for trees of the older classes.

TABLE 1.—*Amount of forking with related data on sample plots*[a] *on the Rochester watershed*[b]

Plot	Trees examined	Age	Trees forked	Trees with precocious buds in terminal 1937 cluster	Trees with secondary lateral near main stem	Average height of stand	Average height of forked trees
	No.	*Years*	*Per cent*	*Per cent*	*Per cent*	*In.*	*In.*
A	417	10–11	92	.....	.....	121	.....
B	100	7–8	63	.....	.....	.....	.....
C	404	6–8	69	.....	.....	27.1	30.0
D	100	5–7	78	30	40	33.1	34.5
E	60	5–7	88	10	38	37.1	37.1
F	88	6–10	92	30	51	.....	.....
G	100	23–25	94	.....	22	324	.....
H	100	7–9	87	41	57	.....	.....

[a] All plots represent different plantings except E and F which are from the same lot.
[b] Blanks in the table indicate that no data were taken.

A map was made of two plots (C and G, Table 1) to determine whether foci of infection occurred in forked stands. The map indicated that forking was evenly distributed throughout the stand, as no evidence of "islands" was found. Two stands of natural red pine within 30 miles of the watershed showed percentages of forking that fell in the range of those of the plantings. In most cases the plantings were of pure red pine, although in some of them there was a light admixture of Norway spruce and eastern white pine, which apparently had no effect upon the prevalence of forking.

The disturbance seems to reach a maximum after a period of years and then gradually subside, although there are indications that there may be a second cycle in the same planting. Annual occurrence of forking when plotted on coordinates (Fig. 3) may be considered additional evidence that an organism was the causal agent of the disturbance.

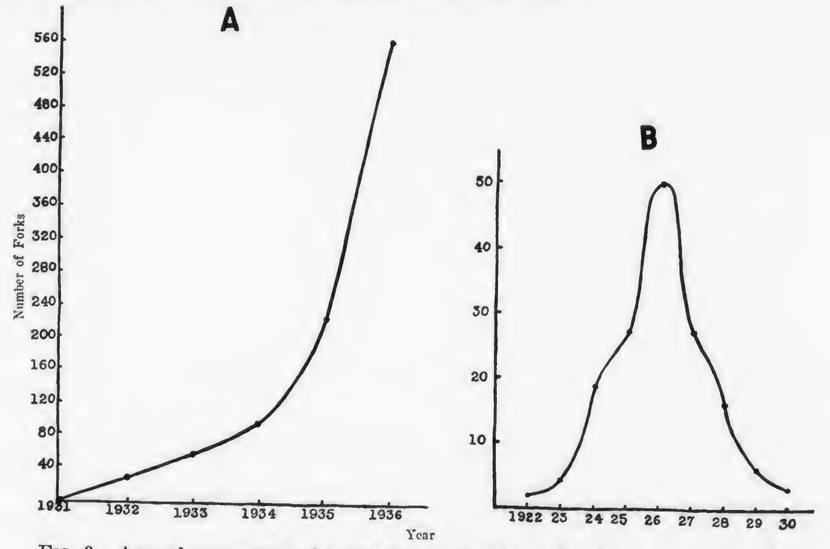


FIG. 3. Annual occurrence of new forks in plantings of red pine. A. Distribution of forks on 852 trees, 6–7 years old, from five plantations. B. Distribution of forks on 100 trees from a plantation in which the trees averaged 23 years old.

HISTOLOGICAL STUDIES

Material for histological examination was fixed in a chromic acid—formalin—salcylic acid solution (2), dehydrated with the N-butyl alcohol technic, and cut on a sliding microtome in sections varying from 5 to 15 μ in thickness. It was found necessary in working with the older tissues to extend the period of dehydration and infiltration to 10 days or 2 weeks in order to insure complete penetration by the alcohol and paraffin. Buds, precocious shoots, wood from the older portions of the trunk, and roots were examined for abnormal anatomical features and for the presence of fungus hyphae. The most successful stain employed was Cartwright's picro-analine blue combination (1), although the chromatic hyphae that appeared in the old wood

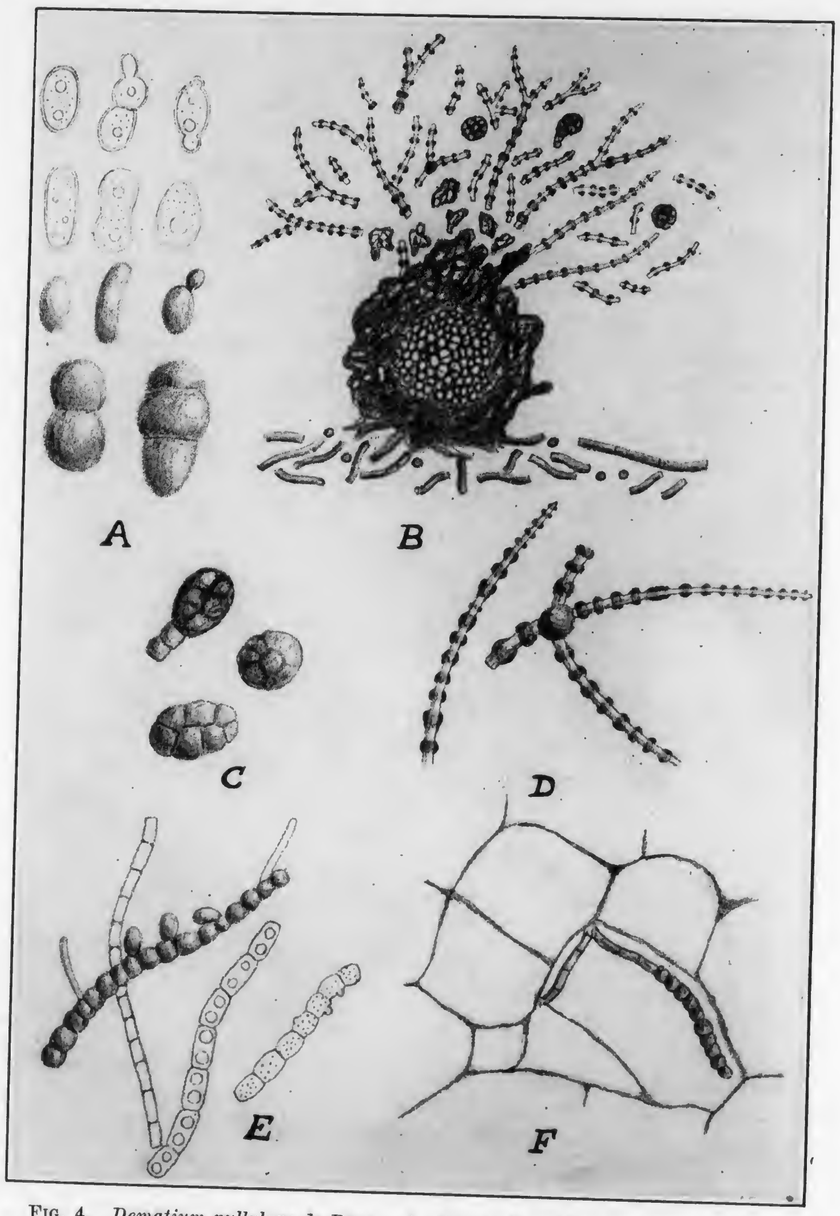


FIG. 4. *Dematium pullulans* de Bary. A. Types of conidia; the spore in the upper left is the typical form. B. Section through a microsclerotium with nodose hyphae and *Coniothecium*-like spores. C. *Coniothecium*-like spores as found among nodose hyphae and in liquid culture. D. Nodose hyphae. E. Types of hyphae. F. Hyphae, believed to be of *D. pullulans* in pith cells of *Pinus resinosa* (drawing from camera lucida).

were satisfactorily demonstrated in unstained sections. No hyphae were observed in the root sections, but chromatic *Torula*-like hyphae were abundant in the pith of the wood taken near a twelve-year-old node (Fig. 4, F) and occasionally penetrated the wood rays. Hyphae occurred both inter- and intra-cellularly in the pith, and were observed entering pith cells through the simple pits in the walls. Minute hyphae also occurred in the procambium and primary xylem of precocious buds, but were not found in the undifferentiated tissue of the promeristem toward the apex of the bud. Such hyphae appeared to be entirely intercellular. There was no evidence of destruction of cells or of the occlusion of vessels in any of the infected material.

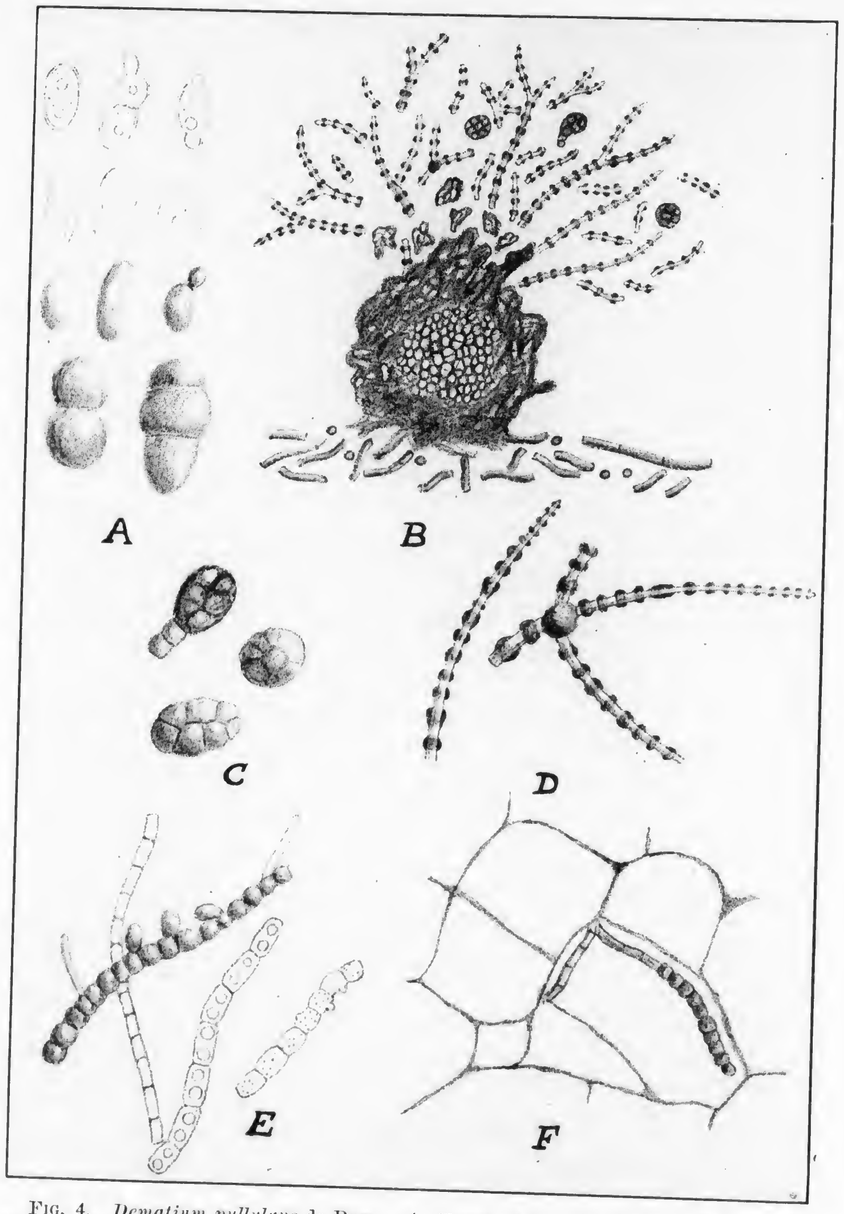
INOCULATIONS

A suspension of conidia of *Dematium pullulans* in distilled water was used to inoculate a series of potted red pines 5 years of age. These trees were brought into the greenhouse from coldframes in early January and inoculated about a week later. Seasonal growth was initiated in the trees, in most cases, by the second week of February. The conidial suspension was introduced into the bud, and into the phloem and pith of the stem by means of a hypodermic needle. Distilled water alone was injected into 3 trees of the series to serve as a control. The site of the needle puncture was ringed with a colored wax pencil, and 5 weeks after inoculation the buds and sections of the stem were surface-sterilized, split longitudinally, and placed in tubes of nutrient agar. Sections of the stem were taken from the site of the inoculation and at points 1 and 2 cm. above and below it. Each tree bore 3 inoculations: in the terminal bud; 1 to 2 in. below the bud; and 8 to 14 in. below the terminal. *D. pullulans* was recovered from all of these sites and, in some cases, from sections 1 and 2 cm. above and below the inoculation.

Another series of potted pines was inoculated by the following method. A small area of the stem was surface-sterilized with 70 per cent alcohol and a slight wound was made in the bark with a sterile needle. This wound penetrated the cells of the cortical parenchyma but did not reach the cambium. A small piece of agar upon which *Dematium pullulans* was growing was placed in contact with the wound; absorbent cotton, wet with distilled water, was placed over it; and a band of cellophane was wrapped around the cotton and tied at both ends to insure a moist atmosphere around the inoculum. The cotton and cellophane were removed after 2 weeks, and five weeks from the date of inoculation an attempt was made to recover *D. pullulans* from the stem in the manner previously described. The failure of *D. pullulans* to develop in any of these cultures leads to the conclusion that it is unable to enter the tree through wounds in the bark or cortical parenchyma. Since resin canals are abundant in the latter tissue, it is possible that the copious resinous exudation may have a fungicidal action.

DEMONSTRATION OF A GROWTH SUBSTANCE PRODUCED BY DEMATIUM PULLULANS

Dematium pullulans when grown in liquid culture produces abundant



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INTENTIONAL SECOND EXPOSURE

growth substance, according to the lupine test developed by Granick and Dunham (4). This technic briefly consists of measuring increase of growth in length of the upper centimeter of a 7–8 cm. etiolated lupine hypocotyl, decapitated at the junction of the hypocotyl and the cotyledons. The substance to be tested is applied to the cut surface in lanolin paste or agar and a mark is made with India ink 1 cm. below the cut. The increase in length of the original centimeter is measured after 4 days exposure to light. For this study *D. pullulans, Tympanis* sp., *Fusarium* sp. and *Polyporus schweinitzii* were grown on liquid 2 per cent malt in 500 cc. Erlenmeyer flasks until a thick hyphal mat covered the surface of the medium. The mat was then removed, washed in water to free it from as much of the malt as possible, and dried thoroughly at a temperature not exceeding 80° C. Fleischman's yeast was grown on Pasteur's solution instead of malt, and freed of the medium by washing in a funnel lined with filter paper. The dried mats were ground in a mortar, and the resulting powder was mixed in a proportion of 2 parts by weight of lanolin to 1 part of the fungus powder. The increase of growth produced by the powder of *Dematium pullulans* was compared with that of other fungi and with control plants upon which lanolin alone was applied. Several series of tests were conducted at intervals of 2 weeks and it was found that the average increase of growth produced by a given organism varied slightly in the different series, but the relative amounts of growth produced by the fungi employed remained constant. This variation might well have been caused by increasing greenhouse temperature and change of season during the experimental period. Fifteen to 30 test plants were used for each species of fungus tested. *Dematium pullulans* produced a greater increase of hypocotyl elongation than any of the other fungi tested. The relative growth-promoting activity of the powdered mats of the fungi may be expressed as follows: *Dematium pullulans* > Fleischman's yeast > *Fusarium* sp. > *Tympanis* sp. > *Polyporus schweinitzii* > controls.

DISCUSSION

The causal agent of forking cannot definitely be determined from the experimental data available at present, but the theory of a fungus origin seems to be the most reasonable. Possible insect injury, genetic factors, or physiological disturbances of a nutritional nature do not appear to provide a satisfactory explanation for the phenomenon.

Complete serial sections of buds were examined, and no traces of larvae or the puncture of insect probosci could be found. There was no evidence of insects on the buds in the course of daily field observations made during late June and the month of July. Even if this evidence were disregarded, an hypothesis of insect injury would have to imply a selective feeding of the organism, since the terminal bud does not ordinarily show extra-seasonal growth. Such a circumstance seems highly improbable.

A physiological disturbance from the standpoint of a nutritional unbalance or excess cannot be correlated satisfactorily with the wide range of soil

types of varying hydrogen-ion concentrations upon which forking occurs. Gustafson (6) found that red pine, brought into the greenhouse in January, would commence growth at once and form 2 bud clusters by July. Apparently the terminal bud develops normally in this case, and there would seem to be no connection with the type of growth that results in forking.

It cannot be proved conclusively that a genetic factor might not be causally connected with forking, but several circumstances are contrary to such a possibility. The wide geographic range of the occurrence of forking, together with the fact that forking in neighboring stands may gradually attain a maximum in different years and at different ages of the plantings (Fig. 3), indicates genetic influence to be unlikely.

The presence of fungi in the buds and internodal pith, as indicated by the results of cultural and histological studies, forms the basis of the writer's suggestion of a theory of the fungus origin of forking. Research on phytohormones has proved that bud inhibition and activation are regulated, at least in some instances, by auxin. It also is well established that certain fungi produce auxin in considerable quantities. Thus, the extra-seasonal growth of laterals might be attributed to the production of auxin within the bud by fungus hyphae. Le Fanu (12) showed that in two shoots of *Pisum,* one of which was being strongly inhibited by the other, the dominant shoot indicated a high concentration of auxin with the *Avena* test, while the inhibited shoot showed only slight traces. If a similar condition occurs in the bud clusters of pine, it would follow that the presence of a phytohormone produced by a fungus in the lateral buds might conceivably act as a stimulus to extra-seasonal growth. Production of the hormone in the terminal bud would not necessarily lead to extra-seasonal stimulation if the hormone were normally present in considerable quantities. The abundant production of growth substance by *Dematium pullulans* and the proof of its ability to inhabit red pine might be regarded as indicative of the possibility that the fungus could be causally connected with the disturbance. It is impossible to compare the microflora of normal trees with those of this investigation. Since forking may be latent in a tree, it could not be determined with certainty that a given specimen was completely normal.

SUMMARY

An abnormal bifurcation of red pine trees is prevalent in New York State and other sections of the northeastern United States. This condition is caused by extra-seasonal shoot elongation in which the lateral buds take part while the terminal remains in the dormant condition. Failure of the inner surfaces of the resulting fork to unite completely during subsequent growth of the tree causes an open fissure in which wood-destroying fungi may enter. *Tympanis* sp. also frequently gains entrance in this manner. No correlation was found between rate of forking and site conditions. Trees at high altitudes in the northern part of the area studied showed the lowest percentage of forking. *Dematium pullulans* de Bary was isolated from

abnormal buds in the majority of cases, and hyphae of the characteristic form of the organism were found in the wood of abnormal trees. Young red pines were inoculated hypodermically with conidial suspensions of *D. pullulans* and, after 5 weeks, the fungus was reisolated from sections of the stem 1 and 2 cm. from the site of inoculation. *D. pullulans* was found to contain a growth promoting substance by means of the lupine technic in a quantity exceeding that of other fungi tested. The theory is advanced that abnormal growth of a pine bud may be initiated through the action of a phytohormone produced by the fungus living within the tissues.

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SOME REACTIONS OF SLIME MOULD PROTOPLASM TO CERTAIN ALKALOIDS AND SNAKE VENOMS

by Samuel S. Lepow

With 3 Text-figures

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INTRODUCTION

The study of the effects or pharmacodynamics of alkaloids and snake venoms has received much attention resulting in a great body of information insofar as organisms and tissues are concerned. The field of protoplasmic pharmacodynamics has received some attention, but there are few data on the effects of snake venoms and alkaloids on protoplasm.

Barber (1911) determined the influence of alkaloids, strychnine, and quinine upon the viscosity of the protoplasm of *Nitella*. Hopkins (1922) investigated the effects of morphine on the swelling and coagulation of the protoplasm of protozoa. These workers did not consider changes in the elasticity, extensibility, tenacity, or colloidal state of protoplasm, qualities to which workers in the field of protoplasm generally attach considerable significance. Balbach (1936) observed the effects of urea solutions on the protoplasm of a slime mould (Chondrioderma difforme)- He noted, among othér responses, granule aggregation, changes in protoplasmic streaming, and swelling or shrinkage of the strands.

The plasmodium of the myxomycete, *Physarum polycephalum*, afforded favorable material for this investigation. A fine pipette controlled by a micromanipulator was used to apply limited quantities of solutions to small areas of protoplasmic strands. The reaction of the treated protoplasm was compared to that of untreated adjacent to it in the same strand (Figs. 3, 9).

Attention was focussed on:

1. The effects of alkaloids and snake venoms on the physical properties of protoplasm.
2. The structure of protoplasm as revealed by these effects.
3. The *modus operandi* of the substances applied.

MATERIALS AND METHODS

The plasmodium of *Physarum polycephalum* was cultured according to the methods developed by Howard (1931) and by Camp (1936). Camp's technique appeared preferable because of the greater facility in culturing, the richer yield of the material and its greater vigor. The organism is grown on powdered oatmeal in a moist chamber. The optimum growth occurred in a water saturated atmosphere at a temperature of 25° C. The original

IRREGULAR PAGINATION

material was obtained from Dr. HOWARD, and has been successfully cultured for four years. The plasmodium, when growing under optimum conditions, easily overcomes and feeds upon molds and bacteria which may appear as contaminants. Ingested ciliates have been observed in vesicles resembling the food vacuoles of protozoa. Cultures showing contaminants were discarded.

Cultures about to change from the active vegetative to the spore or "fruiting" stage were not suitable for study. It was easy to anticipate the fruiting of cultures by noting the tendency of the protoplasm to coalesce into small clumps and the change in color of the agar substratum to brilliant green just prior to fruiting due to acidity, as recorded by SEIFRIZ and ZETZMANN (1935).

A small portion of the plasmodium was removed from the glass wall of the culture chamber and placed on a moist coverslip. After two to four hours, the protoplasm spread into a sheet or a network of strands. Protoplasmic masses which did not spread were discarded. A suitable coverslip-culture was inverted over the micromanipulating chamber, the interior of which was kept moist during the observations. Possible injury by heat from the source of illumination was minimized by the use of a water filter.

The micropipette was introduced into the moist chamber, and varying quantities of the solutions were gently applied to one side of a strand. It was necessary to keep the micropipette from touching the strand and to prevent forceful flow of the solution, since the protoplasm reacts to a purely mechanical stimulus by exhibiting a sudden cessation of motion and a temporary or reversible coagulation. The micropipettes were discarded after use with each individual concentration of the reagent. One to two drops of each sample sufficed usually to produce a result. Strands possessing the least adherent water film were selected in order to prevent dilution of the applied solution.

When fully immersed in water the protoplasm contracted and showed syneresis, therefore the immersion method was not utilized. Injection of the solutions into the plasmodium brought on localized coagulation about the region where the micropipette penetrated and this coagulated material interfered with the flow of the solution into the protoplasm. To overcome this, greater force had to be exerted on the hypodermic syringe. Accurate control of the quantity being introduced could not therefore be attained. The mere insertion of an empty pipette into the protoplasm caused coagulation. BĚLAŘ (1930) noted similar mechanical effects due to micromanipulation. For these reasons microinjection was not continued.

Different concentrations were used in order to determine the toxic range of the substance being studied. The treated protoplasm was observed for at least one hour after the application of the solution so as to observe a possible reversal of pathological changes, slow responses, or other subsequent reactions.

SEIFRIZ (1935) reported that high concentrations of acids and alkalies have a pronounced effect on the plasmodium of *Physarum polycephalum*, and CRANE (1921) found that alkaline solutions of alkaloids are more toxic than acid solutions for Paramecium. A series of studies were, therefore, made with HCl solutions of different pH values. It was found that H^+ within a range of 4.0 to 7.0 exerted no effects comparable to those obtained with the toxic solutions with pH values from 4.1 to 7.1, epinephrine being the only exception with a pH of 3.0. Buffers were not used because of toxic effects.

Observations were made on strands of varying thickness, size, and rate of streaming in order to eliminate misinterpretation. Strands showing signs of degeneration were not used.

NORMAL SLIME MOULD PROTOPLASM

A plasmodium is a mass of relatively undifferentiated protoplasm. The plasmodium of *Physarum polycephalum* is bright lemon-yellow in color. SEIFRIZ and ZETZMANN (1935) showed that the pigment responsible for this color is an indicator of pH. The pigment is present in granules and not dissolved as in colloidal suspension in the matrix, for all non-granular areas are colorless. The protoplasm contains pigment granules, oil droplets, and other globules with well defined contours including some few colorless vesicles resembling oil globules and probably of the nature of vacuoles, all of which may collectively be termed macrosomes. With these occur minute particles or microsomes.

The protoplasmic stream is in one direction only at a time and reverses itself repeatedly.

The viscosity of the quiescent protoplasm is that of an elastic gel whereas the flowing protoplasm is liquid. The elasticity (that property of protoplasm by virtue of which it tends to recover its original form after subjection to a distorting force) is low, while extensibility (stretch as distinct from elasticity) is great or low depending on the size of the strand and condition of the protoplasm. The tenacity or cohesion of the protoplasm as determined by the ease with which it can be torn is moderate, but varies somewhat.

The criteria used to indicate toxicity were:

(1) Effects on streaming
(2) Swelling and shrinkage
(3) Changes in color
(4) Coagulation
(5) Brownian movement
(6) Syneresis
(7) Outflow of matter
(8) Changes in physical proporties
(9) Reversibility of changes

COMPOUNDS USED[1]

(a) *Alkaloids*
Cocaine hydrochloride
Quinine hydrochloride
Quinine sulphate
Atropine sulphate
Morphine sulphate
Ephedrine hydrochloride
Strychnine sulphate

(b) *Venoms*
Bothrops atrox
Crotalus atrox

(c) *Other substances*
Chloral hydrate
Epinephrine hydrochloride

EXPERIMENTAL RESULTS

The following results are general for all solutions used except where a specific reagent is named.

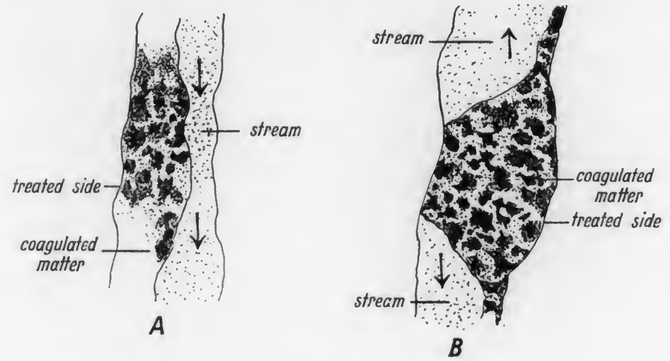
Effects on streaming

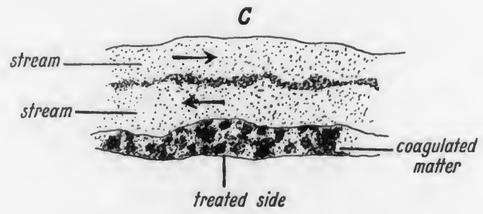
The initial effect on streaming was a temporary stimulation resulting in an acceleration of the rate. The subsequent reaction varied. Quinine hydrochloride caused complete cessation of motion a few seconds after the initial stimulation. Morphine eliminated all motion in two to three minutes. When streaming was not abolished in five minutes, no reaction to the added solution could be observed. Frequently, movement would continue at a reduced rate on the untreated side of the strand until the reagent had penetrated this region

[1] Dr. SCHMIDT, of the Department of Pharmacology, School of Medicine, University of Pennsylvania very kindly furnished the alkaloids used in this investigation. The venoms were obtained through the kindness of Dr. GITHENS, of the Sharp and Dohme Biological Station.

IRREGULAR PAGINATION

(Fig. *a*). At times the protoplasm would draw away from the coagulated site and then produce two streams which flowed in opposite directions but did not show reversal (Fig. *b*). Often the protoplasm would move in jerky spurts as if encountering a barrier, rebounding and then pushing forward. An unusual response was noted in one strand (Fig. *c*) in that the actively flowing protoplasm became less opaque, due apparently to precipitation of some of the protein; as shown by coagula which appeared within the streaming protoplasm. Another type of flow occurred especially after treatment with cocaine which is of quite a different kind from the normal flow. The hyaline substratum moved very sluggishly in an irregular path through the coagulated, granular mass. The movement does not undergo reversal and appears to be due to mechanical pressure, possibly shrinkage of the protoplasm on coagulation.





SWELLING AND SHRINKAGE

Swelling is to be distinguished from normal enlargement which occurs with growth and movement; the latter is reversible, while swelling is permanent. With increase in volume due to a pathological change, there is no recovery, no return to the original volume after swelling (Figs. 8, 18). Abnormal swelling is characterized by the presence of hyaline, granule-free areas at the treated site.

Shrinkage was caused by the removal of protoplasm from the treated region to adjacent untreated regions, and by contraction of the coagulated protoplasm accompanied by the production of syneretic vesicles (Fig. 16).

CHANGES IN COLOR

The yellow pigment of Physarum changes to yellow-green with alkalinity and to orange-red with acidity. Epinephrine with a pH of 3.0 caused the color to change to orange, i. e. to the acid side. Cocaine and several of the other reagents altered the color to a greenish yellow indicating an increase in acidity.

Other changes in color occur which are not due to the pigment, but to aggregation of granules, swelling, and shrinkage which alter the depth of color or opacity.

Coagulation

The coagulation of protoplasm concerns primarily the proteins dispersed in the aqueous substratum, but agglutination or aggregation of particles may occur with the coagulation of the proteins.

Any reagent which has an ill effect on protoplasm is likely to cause coagulation. Coagulation is most readily recognized by the aggregation of the visible protoplasmic particles. Cocaine causes aggregation of the particles into one irregular mass (Fig. 8).

A coarsely granular coagulum due to aggregation of particles into large and scattered groups was produced by quinine sulphate (Fig. 17), atropine sulphate (Fig. 21), and ephedrine hydrochloride (Figs. 19, 22). A moderately coarse coagulum of aggregates in uniform distribution was caused by epinephrine hydrochloride (commercial solution).

A finely granular coagulum was produced by strychnine sulphate (Fig. 6), chloral hydrate (Figs. 14 and 20) and the venom of *Bothrops atrox*. A very finely granular almost homogeneous appearance due to aggregation of particles into very small groups was produced by quinine hydrochloride (Fig. 4) and U. S. P. epinephrine (Fig. 1). *Crotalus atrox* venom did not appear to modify granule dispersion or to profoundly influence the protoplasm in any way (Fig. 15).

BROWNIAN MOVEMENT

Brownian movement of microsomes was not visible in streaming portions of the protoplasm but could be seen when streaming ceased if phase separation occurred. The degree of fluidity was roughly indicated by the amplitude of vibration of the microsomes. The duration of the movement varied with the substance used (cf. table I). Brownian movement was not obersved when coagulation took place without phase separation. The granules did not undergo Brownian movement. Although phase separation was a common reaction, the vibration of the microsomes failed to occur in every instance.

SYNERESIS

The squeezing out of a fluid with the contraction of a gel is known as syneresis. It occurs in protoplasm on coagulation (Table I). Syneresis assumed several forms:

1. Vesicles which contained a hyaline fluid only (Fig. 16*a*, 22*a*).
2. Vesicles containing hyaline fluid and other protoplasmic constituents (Figs. 2*a*, 7, 10, 15*a*, 16, 22).

3. Irregular extensions of hyaline fluid and protoplasmic inclusions (Fig. 6).
4. Peripheral extensions of hyaline fluid.

Syneresis is an entirely different response from that which is here termed outflow (cf. Fig. 22*a* and 22*b*).

OUTFLOW OF MATTER

Normal protoplasm may rupture the surface and produce an outflow as a result of pressure created by a stream block. This matter undergoes reversible coagulation, recovers and produces a new surface. There is no separation of the protoplasm into several phases. Quinine sulphate caused a different type of outflow which appeared to be a toxic effect. The surface possessed a ragged opening which was probably caused by death changes and loss of elasticity. This outflow consisted of hyaline fluid and microsomes. There was, therefore, a protoplasmic separation in this type of outflow. The microsomes were undergoing Brownian movement. Later, the microsomes aggregated and the hyaline fluid was found to be miscible with the aqueous solution on the coverslip. Ephedrine and atropine also produced an outflow.

CHANGES IN PHYSICAL PROPERTIES

a) Viscosity

Viscosity changes were caused by all the reagents. The outer treated regions were affected more than the inner ones. The changes differed with each reagent (cf. quinine hydrochloride and atropine, Table I).

b) Elasticity

The elasticity generally increased on application of the toxic substances, but it was to be observed only after the protoplasm underwent structural changes.

c) Extensibility

An increase in extensibility due to treatment was noted in protoplasm which had not coagulated. Quinine hydrochloride produced a rather uniform coagulum which possessed little extensibility.

d) Tenacious qualities

There was an apparent relationship between extensibility and tenacity. A coagulum was rather difficult to tear and scarcely stretched. Protoplasm which had not coagulated throughout was less difficult to tear, the torn surface was not smooth and the extensibility was increased.

SPECIFIC EFFECTS OF REAGENTS

Cocaine hydrochloride

Sollman (1936) described cocaine as a typical if rather weak, protoplasmic poison, paralyzing all sorts of cells, *without producing* any gross chemical changes. Cocaine in high concentrations, from 1—10 caused, first, liquefaction, and later

gelation. This resembles the death change in protoplasm reported by Chambers (1917). The co-existence of apparently normal protoplasm side by side with dead matter was seen after cocaine had acted (Fig. 8). A mechanical barrier appeared to separate these regions. A 1—1000 solution produced a reversible coagulation, which suggests the hypothesis of Bernard (1875) namely, that narcosis is a reversible coagulation of protoplasm.

Fig. 1. Effects of *Bothrops atrox*, 1—20.
a. Treated strand showing granule aggregation, hyaline areas and swelling.
b. untreated strand (the control).

Fig. 2. Effects of morphine sulphate, 1—20.
a. Treated strand showing swelling, slight syneresis and coarse granular appearance resulting from coagulation and granular clumping.

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INTENTIONAL SECOND EXPOSURE

b. untreated control.
c. granular periphery produced by surface effects.

Fig. 3. Treated and control in a single strand.
a. reversible coagulation of peripheral regions.
b. untreated side of strand which is normal.

Fig. 4. Effects of quinine hydrochloride, 1—150.
a. untreated control.
b. treated strand showing the coagulum and relatively uniform distribution of small granular aggregates.

Fig. 5. Effects of an old commercial epinephrine hydrochloride solution, 1—1000.
a. living protoplasm.
b. protoplasm showing fibrillar structure.

Fig. 6. Effects of strychnine sulphate, 1—40.
a. irregular syneretic areas containing microsomes and closely packed granules due to contraction of gel.
b. phase separation, showing coagulum and fluid hyaline areas.

Fig. 7. Effects of chlorbutanol, 1—125.
a. dead matter showing granule aggregation and syneretic vesicles containing microsomes and larger granules.

Fig. 8. Effects of cocaine hydrochloride, solution, 1—10.
a. treated strand showing swelling, phase separation and granule aggregation.
b. living protoplasm.
c. barrier separating dead and living regions.

Fig. 9. Effects of resorcinol, 1—10.
a. treated strand showing coagulum and granule aggregates.
b. normal protoplasm.

Fig. 10. Degeneration following application of an old commercial epinephrine, 1—1000. note syneretic vesicles, contracted gel and massed granules.

Fig. 11. Portion of a normal strand for comparison.

Fig. 12. Effects of epinephrine hydrochloride U. S. P. standard, 1—1000: granules aggregated into small groups and dispersion medium altered in appearance.

Fig. 13. Peculiar effects of *Crotalus atrox* venom, 1—200.
a. dead matter alternating with knots of living matter (*b*).
c. venom particles.

Chloral hydrate

This compound was the only substance of synthetic origin used in this investigation; it is a nerve depressant and hypnotic. Chloral hydrate was used in concentrations ranging from 1—10 to 1—300. An acceleration of streaming with no other apparent toxic effects was produced by a 1—300 solution. Although cocaine and chloral hydrate are both nerve depressants, their effects on slime mould protoplasm are not identical.

Quinine

Dixon and de Premankur (1927) working with *Paramecium*, concluded that all quinine derivatives are protoplasmic poisons.

a) *Quinine hydrochloride*

Quinine hydrochloride was distinctive in producing a more rigid coagulum than did any other reagent used. Other general effects of this reagent are given in Table I.

b) *Quinine sulphate*

This substance was used to compare the toxicity of different salts of the same alkaloid. The results indicate that the sulphate, in the same concentrations as the hydrochloride, is less toxic (Table I). Several explanations are:

1. The hydrochloride contains from 7 to 8 per cent more of the quinine radicle, by weight.
2. The different anions may have modified the effect: see Kahho (1921), Reznikoff and Chambers (1927).
3. The dissociation constants of the salts differ.

Strychnine sulphate

This alkaloid has been described as a weak protoplasmic poison on amoebae and yeasts. A 1—100 solution was found to be scarcely toxic. Strychnine, compared with the other alkaloids, is destructive to slime mould protoplasm only at relatively high concentrations (1—40). Syneresis is particularly characteristic of this compound (Fig. 6).

Morphine sulphate

This alkaloid has been said to be one of the weakest in its action. A concentration of 1—100 produced no apparent effects. So high a concentration as 1—20 was not lethal. Data suggesting a fibrous structure for protoplasm were found in the following observations: The protoplasm, adjacent to the treated area, moved away and hyaline fibril-like structures with the granules arranged in a linear fashion were produced (Fig. 18). This behavior was also noted with untreated protoplasm and can scarcely be credited to toxic effect. The response is apparently an evidence of irritability.

Atrophine sulphate

Atropine in a concentration of 1—500 did not give rise to any apparent effects. More concentrated solutions, to 1—100, produced a hyaline band along the periphery of the protoplasm which appeared to result from syneresis. Atropine-treated protoplasm failed to coagulate completely; the outer regions possessed high viscosity whereas the internal matter had but moderate consistency. Outflow of the protoplasm occurs as the result of a break at the surface.

Ephedrine hydrochloride

Atropine and ephedrine are grouped together by pharmacologists. Ephedrine in a concentration of 1—500 had no pronounced influence beyond a slight temporary reversible effect on streaming. A 1—1000 solution caused no apparent effects. Ephedrine was similar to atropine in its influence on protoplasm (Table I) Figs. 19, 21, 22). In comparable solutions (1—200 ephedrine influenced the inner protoplasm in such a manner that the microsomes failed to exhibit Brownian movement — indicating high viscosity — but atropine-treated protoplasm showed this motion — indicating low viscosity.

Table

Substance used	Minimum lethal concentration	Effect on streaming	Swelling (sw.) and shrinking (sh.)	Phase separation	Effect on Brownian movement of microsomes
Cocaine hydrochloride	1—1000	temporary stimulation, then cessation except for trace	sw.	+	none
Chloralhydrate	1—200	temporary stimulation, then cessation	sw.	+	none at 1—10 active at 1—200
Quinine hydrochloride	1—500	ceased after several seconds	sw.	trace	
Quinine sulphate	1—250	ceased after one minute	sw.	+	active
Strychnine sulphate	1—100	ceased after one minute	sw.	+	slight
Morphine sulphate	1—75	cessation except for sluggish trace	sw.	+	active
Atropine sulphate	1—250	ceased after one minute	sw.	+	active
Ephedrine hydrochloride	1—300	ceased after one minute	sw.	+	active
Bothrops atrox venom	1—300	decreased, then stopped after one minute			
Crotalus atrox venom	1—10	decreased, but did not cease, later recovery	slight peripheral swelling		

Epinephrine hydrochloride

a) Commercial 1—1000 solution

This solution contained chlorbutanol, sodium sulphite and physiological salt solution. The pH of the preparation was approximately 3.0. Since this solution contained other compounds it was not possible to attribute the protoplasmic response solely to the epinephrine. The results are given to note the unusual reactions.

A response occurred after 30 seconds. Streaming ceased and syneretic bubbles appeared. The protoplasm showed no outflow when torn and possessed moderate elasticity. Granules became arranged in compact groups. Five minutes later the strand was shrunken and the syneretic areas were considerably larger (Fig. 16).

Experiments with the anti-oxidants and preservatives (e. g., chlorbutanol) present in this solution showed each to be toxic, but in a way different from the original solution (Fig. 7). The effects were chiefly synergistic ones.

I

Color changes	Syneresis	Viscosity effects	Effect on elasticity	Effect on Extensibility	Influence on tenacity
Lighter	slight hyaline vesicles	highly viscous gel	increase	increase	more tenacious
Lighter	slight	consistency of bread dough	increase	slightly extensible	increased tenacity
Opaque; brownish	slight	rigid gel	slight increase	scarcely extensible	highly tenacious
Opaque; brownish	moderate	consistency of butter	increase	slight increase	somewhat increased
Opaque; brownish	considerable	increase in viscosity	increase	no change	more tenacious
No change		slight increase in viscosity	considerable increase	increase	increase
Darker		slight increase in viscosity			less tenacious
Lighter	syneretic vesicles	outer region a gel; inner of moderate viscosity	increase	slight increase	less tenacious
Lighter		gelation	slight increase	slight increase	less tenacious
No change		partial coagulation			

b) Epinephrine hydrochloride 1—1000 U. S. P.[1]

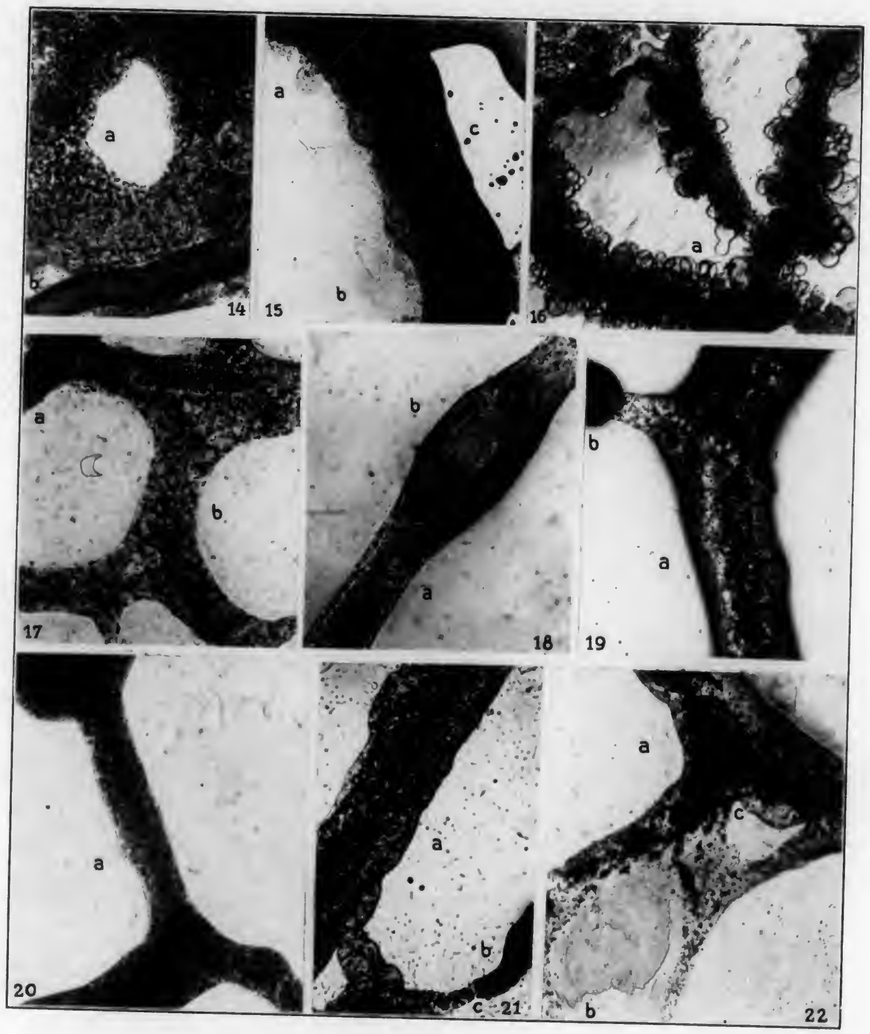
This solution was not made with the preservatives, etc. of the commercial solution. The nature of the specific effects of epinephrine remained unknown because this substance in the crystalline state is basic and must be converted into the hydrochloride to be made soluble. The pH of the solution was 3.0 and therefore an H^+ effect occurred (cf. Methods). The results with this solution are in sharp contrast with those of the other (commercial) sample. First acceleration and then a cessation of streaming occurred. The color became orange, indicating a lowered pH of the protoplasm. Granules were grouped into small aggregates (Fig. 12). The strand tore easily. Elasticity and extensibility were slight. These properties are similar to the acid effects noted by Seifriz (1936).

[1] The standardized crystalline epinephrine was supplied by Dr. E. Fullerton Cook, of the Philadelphia College of Pharmacy and Science.

Venoms

a) Bothrops atrox

Venoms produce a colloidal suspension when placed in distilled water. *Bothrops atrox* caused a transitory stimulation in streaming rate when at a concentration of 1—1000. The treated region was abandoned by the streaming protoplasm after twenty minutes. A 1—200 suspension produced a reaction similar to that reported in table I with the exception that traces of recovery were noted in isolated spots where the injury was apparently of slight consequence. More concentrated suspensions produced irreversible changes.



b) Crotalus atrox

Essex and Markowitz (1930) stated that this venom is a specific protoplasmic poison which affects protoplasm wherever it contacts it. A 1—20 suspension slowly influenced the periphery, the protoplasm becoming less active although streaming did not cease (Fig. 15). The treated regions were eventually abandoned by the active protoplasm. A peculiar reaction occurred in several experiments. There were alternating areas of apparently normal and dead protoplasm in the same strand (Fig. 13). This may be a different type of adjustment to the presence of injured protoplasm.

It was not possible to note any pronounced changes in physical properties where streaming had not ceased, but in the abandoned areas the protoplasm was of butter-like consistency, scarcely extensible, showed little tenacity, and was moderately elastic.

Description of Figs. 14 to 22 (p. 172)

Fig. 14. Effects of chloral hydrate, 1—10.
a. mottled coagulum resulting from granule clumping with hyaline areas.
b. an "artery" of streaming protoplasm: note granule aggregation on side bordering dead matter.

Fig. 15. Effects of *Crotalus atrox* venom, 1—20.
a. vesicular outflow (membrane unruptured) consisting of granules and microsomes.
b. matter from a ruptured vesicle.
c. streaming protoplasm.

Fig. 16. Effects of a fresh 1—1000 commercial epinephrine hydrochloride solution.
a. hyaline syneretic vesicle: note contraction of strand and close packing of granules.

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a. normal protoplasm.
b. large granular aggregates, syneresis, and phase separation.

Fig. 18. Strand treated with resorcinol, 1—10.
a. living matter showing fibrillar structure.
b. swelling and phase separation.

Fig. 19. Effects of ephedrine hydrochloride, 1—10.
a. phase separation and large granular groups.
b. living matter.

Fig. 20. Effects of chloral hydrate, 1—10.
a. slight swelling, hyaline were area and mottled appearance.

Fig. 21. Effects of atropine sulphate, 1—10.
a. large granular aggregates and phase separation.
b. living matter.
c. separation between dead and living regions.

Fig. 22. Effects of ephedrine hydrochloride, 1—10.
a. hyaline syneretic vesicles.
b. hyaline residue outflow with microsomes.
c. phase separation.

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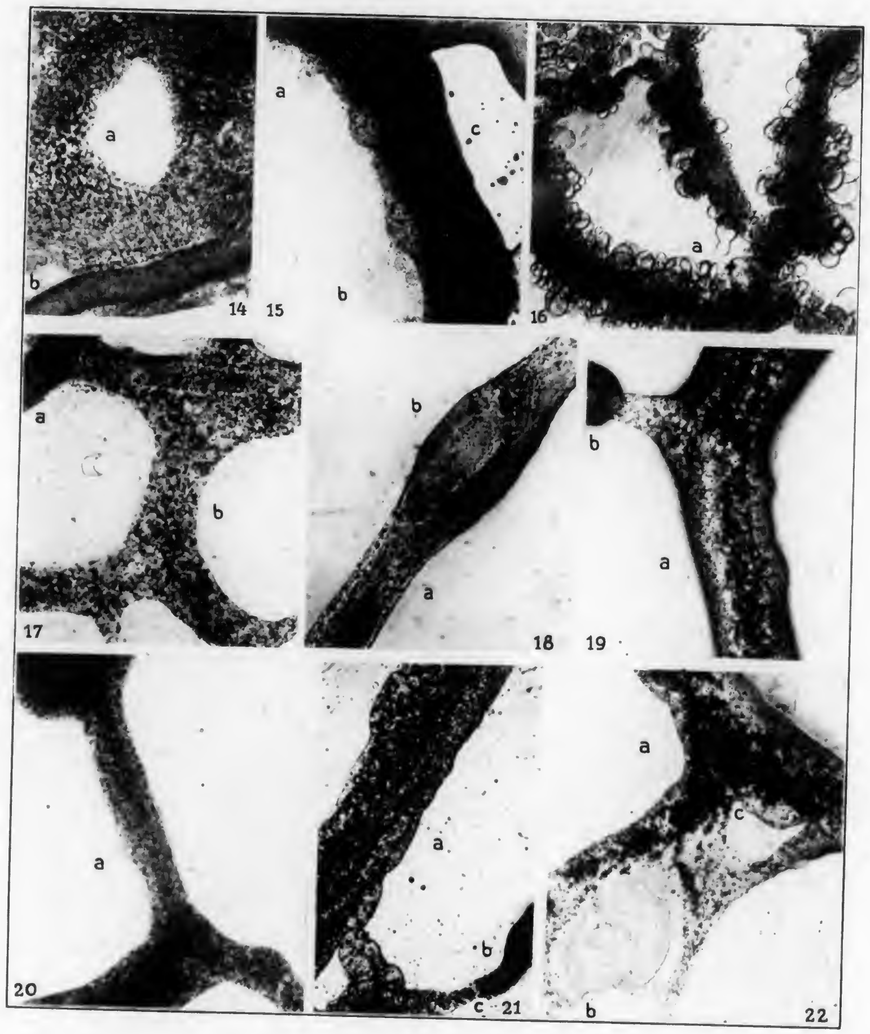

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b. hyaline residue outflow with microsomes.
c. phase separation.

INTENTIONAL SECOND EXPOSURE

DISCUSSION

a) Comparison of toxicity

The greatest toxic effect on any reagent is evident by the production of a coagulum which fails to show phase separation. The latter response results when less concentrated solutions are used. Quinine hydrochloride appears to be the most toxic alkaloid since it produced the greatest toxic effect at a comparatively low concentration. There is no relationship between toxicity and molecular weight of the poison. Members of the same group did not cause parallel degrees of destruction (e. g. the quinoline group, quinine and strychnine). Very similar effects were produced by atropine and ephedrine which belong to different chemical groups. The degree and type of dissociation is an important factor in toxicity of alkaloids according to Botazzi (1928). He noted that toxicity of alkaloids depends on particle size and the presence of undissociated molecules. While quinine is more destructive in its effects on protoplasm, and therefore in this sense more toxic, cocaine produces harmful effects at much lower concentrations (e. g. 1—10,000 as compared with 1—500 for quinine). The morphine effects compared favorably with the data of other workers in being the least toxic of the alkaloids investigated.

Slime mold protoplasm is more sensitive to the venom of *Bothrops atrox* than to that of *Crotalus atrox*. Philpott (1930) showed the same to be true for *Paramecium*. Githens (personal communication) noted similar results for the pigeon.

Slime mold protoplasm is more sensitive to the alkaloids than to the venoms. Several possible reasons are:

1. The mechanism of action differs.

2. The venoms were used in the crude state and the concentrations were therefore not comparable.

3. If slime mold protoplasm is animal-like, then the suggestion of Macht (1930), that poisons of plant origin are more toxic for animals than poisons produced by plants, may be the explanation.

4. Slime mold protoplasm contains cholesterol (Seifriz, 1936), Phisalix (1897) observed that cholesterol exerts a detoxifying action on snake venoms.

The last two suggestions re-emphasize a principle established by True (1930) for plants; namely, that the protoplasms of different species have different toxic equivalents because they are chemically dissimilar.

b) Effects on protoplasmic structure

The most significant structural change in this investigation is the separation of the protoplasm into the coagulated phase and the hyaline, fluid phase. Addoms (1927) reported Brownian movement in clear spaces between flocculent masses. This response closely resembles phase separation. Lepeschkin (1927) noted that poisons act on the dispersed protein phase when coagulation occurred. Schackell (1923) investigating protoplasmic poisoning by phenols, assumed that a factor in the maintenance of dispersion of tissue proteins was the adsorption of lipins at the water-protein interface, the inference being that substances which interfere with the adsorption of these lipins would permit change in the dispersion of tissue proteins and therefore would be toxic.

Since coagulability is a characteristic of proteins and as these constitute the major portion of the dispersed phase, the occurrence of coagulation in protoplasm indicates a change in the dispersion of proteins, an alteration in protoplasmic structure, changed physical properties, and resulting toxic effects. The coagula may, as has been noted, be of several types.

Changes in pH were detected by differences in color of the pigment. The change was in all cases an increase in acidity which might result from the acidity of the solution, from injury, or from the reaction of the protoplasm to the solution. Changes in pH are indicative of physical and chemical effects; therefore it appears that the view of Sollman regarding cocaine is untenable because he stated that this alkaloid produces no gross chemical effects.

Seifriz (1918) pointed out that normal protoplasm is not miscible with water but that degenerated living matter is miscible. Chambers (1928) observed the dissipation of the inner matter of Echinoderm eggs in the surrounding medium. The evidence obtained from abnormal outflow in slime molds indicates that the dispersion medium of protoplasm is an aqueous solution. There was further no evidence to indicate phase reversal such as occurs with emulsions.

c) Changes in physical properties

Structural changes should result in other altered physical properties. Chambers (1927) noted that injury is accompanied by swelling and an apparent increase in the acid reaction of the part involved. Furthermore, when the protoplasm becomes disorganized the liquid hyaloplasm may flow out and disappear in the surrounding medium or it may set and form a rigid, coagulated mass. Similar results were observed with quinine sulphate and ephedrine in this investigation. These structural changes influenced the physical properties as recorded in Table I. Seifriz (1920) observed changes in consistency following chemical and mechanical injury and emphasized the coarsely granular appearance of the coagulated protoplasm. The present investigation has produced results paralleling those of Seifriz and further indicate the influence of altered structure on the physical properties. Heilbrunn (1920) stated that viscosity changes are associated with structural changes and cell metabolism.

Brownian movement indicates a fluid protoplasm and the greater the amplitude of vibration the lower the viscosity. Bayliss (1920) observed the sudden cessation of Brownian movement of the particles in the pseudopodia of amoebae following electrical stimulation and concluded that a reversible change from sol to gel state had occurred. Addoms (1927) observed that Brownian movement diminished as coagulation and flocculation proceeded. That cessation of Brownian movement of microsomes indicates an increase in viscosity in slime mold protoplasm was verified by micromanipulation.

Changes in elasticity and extensibility are probably due to structural modifications. Different types of coagula possess different elastic and extensile

values. SEIFRIZ (1920) noted that the protoplasmic coagulum is not as extensible as living matter. The tenacity and the type of tearing are related to structure. A true coagulum is difficult to tear and stretches but slightly. Protoplasm treated with quinine hydrochloride is slightly extensible, while the atropine, ephedrine, and cocaine treated matter is not difficult to tear, and the torn surface is ragged, but the stretch is greater. All these properties of protoplasm depend upon structure.

d) Protoplasmic structure as revealed by the effects

SEIFRIZ (1936a) describes protoplasm as consisting of three types of systems, an emulsion, a true solution and a gelforming system, all three distinct as to kind but intimately associated. Coagulation is evidence of the presence of a gelforming system. Syneresis adds further evidence. Miscibility of the hyaline residue with the applied aqueous solution indicates the aqueous nature of the dispersion-medium.

When slime-mold protoplasm leaves a treated region it forms on stretching fibrills containing granules arranged in a row and apparently imbedded (Figs. 5, 18). This arrangement was also seen in normal protoplasm. This is suggestive of the statement by SEIFRIZ (1926) that protoplasm is essentially protein and fibrous in nature.

The composition, location, and function of the pigment in slime-mold protoplasm are not fully understood. This investigation reveals that the pigment is associated with the granules because no granules were found in hyaline areas. The color was always intensified when granule aggregation occurred.

e) Mechanism of action of the toxic substances

The results demonstrate differences in degree of toxic effect with various concentrations and a minimum concentration is necessary for effects to occur. In adsorption, the amount of a substance taken up is in proportion to the concentration and a certain minimum is required in order that the quantity necessary to produce a reaction is adsorbed (BAYLISS 1924). SCARTH and LLOYD (1930) point out that alkaloids in aqueous solutions are surface active and highly adsorbed. They further note that there is a relationship between toxic action and surface activity and suggest that alkaloids act through adsorption.

Swelling appeared to be due to surface effects. Adsorption accompanied by a lowering of surface tension would reduce the restraining force of the membrane and permit an inner pressure to advance the surface.

LECOMTE DU NOÜY (1936) noted that adsorption was far from being immediate or even very rapid in effect, but could be measured from time to time by changes in surface tension. CLARK (1933) believes that rapidly acting drugs could scarcely penetrate the cell membrane in three or four seconds and that the reaction is probably a result of surface effects. HÖBER (1926) stated that the results of the action of salts on cells would be a change in the consistency and dispersion of colloids and that the changes which the surface colloids undergo are of great importance.

The above statements recognize the significance of surface reactions. Further support is to be had from the fact that injection produced lesser effects than did external application because of the absence of surface effects. It is possible that the alkaloids have an electrostatic effect because of adsorption on the negatively charged protoplasmic surface. ADDOMS (1927) suggested that different constituents of protoplasm may adsorb different cations in varying degrees. This may explain the variation in toxicities.

While the chief harmful effects appear to be the result of surface reactions, yet the substances used in the present experiments do enter the protoplasm after the surface effects have occured. The untreated side of a strand was affected some time after the treated side.

SUMMARY

1. The protoplasm of the slime-mold *Physarum polycephalum* was treated with seven different alkaloids, two snake venoms, epinephrine, and chloral-hydrate.

2. Micromanipulation was employd as an aid in ascertaining the effects of the foregoing substances on protoplasm.

3. The following effects were observed:

a) Streaming was greatly influenced in all cases, a complete cessation of motion with no recovery being the usual result.

b) Pronounced swelling followed application of the toxic substances in most cases.

c) Separation of the protoplasm into a coagulated phase and a hyaline, fluid phase, was the outstanding structural change.

d) Brownian movement of the microsomes was occasionally observed in hyaline regions indicating low viscosity for these areas.

e) The pigment, which is a pH indicator, underwent color changes indicating change in acidity. Usually the protoplasm became more acid.

f) Syneresis, in various forms, frequently occurred.

g) The viscosity of the granular plasm increased usually to a rigid coagulum but the dispersion medium or matrix often remained fluid.

h) Elasticity and extensibility of the protoplasm were generally increased.

i) Tenacity was increased by some substances and decreased by others.

4. The toxic effects recorded appear to be due to structural changes in the protoplasm.

5. Quinine was found to be the most toxic of the alkaloid group.

6. The venom of *Bothrops atrox* was more toxic than that of *Crotalus atrox*.

7. Molecular weight was not a factor in toxicity: degree of dissociation is suggested as a possible factor.

8. Surface change due to adsorption of the poisonous agent is suggested as the chief factor in toxicity.

9. The observations indicate that slime mold protoplasm consists of an aqueous dispersion medium and a dispersed gelforming phase, the latter of fibrous structure.

10. The pigment in this organism is associated with the granules.

11. The results indicate that the micromanipulation of slime mold protoplasm treated with toxic agents should be of value in pharmacodynamic studies.

ACKNOWLEDGMENTS

I wish to express my deepest appreciation to Professor WILLIAM SEIFRIZ for his critical supervision, impersonal criticism, and unfailing interest. I am also indebted to Professor RODNEY H. TRUE for extending to me the facilities of the micromanipulation section of the Botanical Laboratories.

Botanical Laboratories University of Pennsylvania Philadelphia, Pennsylvania.

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EFFECTS OF X-RAYS ON *ZEA MAYS*

MARY A. RUSSELL

(WITH EIGHT FIGURES)

Introduction

Since the discovery of x-rays, their effects on both plants and animals have been studied as important biological problems. Many reports of early work are conflicting because quantitative results have been possible only in recent years, after methods of accurately measuring the dosage were perfected.

In the study of many general problems, plants are ideal material because of the large numbers which can be used and the ease with which environmental conditions can be modified or kept constant as occasion demands. Samples can be taken for cytological studies as the experiment progresses without detracting from the final results. Numerous reports on plant experiments have come from hospital laboratories where the work has been done with the conviction that many of the results will be applicable to problems of animal x-ray technique.

The work reported here was started for the purpose of finding out how *Zea mays* would react to x-ray treatment in various aspects of its growth and appearance, in order to use it eventually as a test material for studying effects of irradiation as they might be influenced by changing environmental conditions. *Zea mays* has been used occasionally for special radiation problems. STADLER (**14**) found it valuable for studies of mutations produced by x-rays because its genes had been more thoroughly mapped than those of many other plants. BERSA (**2**) reported *Zea mays* to be relatively more resistant to irradiation than many plants which he tested. He used it extensively for cytological examination of irradiated root tips.

Materials and methods

The source of radiation was a standard air-cooled Coolidge tube. A constant potential of 180 kv. was used and the filament current was maintained at 3.7 ma. The distance from the center of the target to the material to be irradiated was 30 cm. The only filters were the heavy pyrex glass of the tube and the paper over the seedlings. The actual strength of the x-rays used was measured during radiation by the usual ionization-galvanometer arrangement, the ionization chamber having been calibrated previously with a standard chamber as described by TAYLOR and SINGER (**17**). The calibration of the apparatus gave a factor of 1.7 r units per centimeter of deflection of the galvanometer. Thus at a deflection of 15 cm. for 40 minutes, the dose

IRREGULAR PAGINATION

would be $1.7 \times 15 \times 40$ or 1020 r. The ROENTGEN unit or "r" was defined at the Second International Congress of Radiology in 1928 as follows: "... the quantity of radiation which, when the secondary electrons are fully utilized and the wall effect of the chamber is avoided, produces in one cubic cm. of atmospheric air at 0° C. and 76 cm. mercury pressure such a degree of conductivity that one electrostatic unit of charge is measured at saturation current" (**16**).

Improved Golden Dent field corn was used in all experiments. The dry grains were planted between layers of moist paper toweling in glass chambers placed in a dark incubator at 24° C. After 72 hours most of the primary roots were slightly more than 1 cm. long. The grain was always placed in the germinating chamber in the evening at about the same hour in order to avoid differences in sensitivity due to possible natural mitotic rhythm. These precautions were also for the purpose of obtaining an equal degree of hydration in all experiments.

Seedlings were selected which had fairly straight roots 10 to 15 mm. long. A spot of India ink was placed exactly 10 mm. from the tip of each root and all future measurements were made from this point. In most experiments 20 seedlings were used in each group. The plants to be treated were individually placed on damp filter paper over the bottoms of glass dishes. The side of the grain where the shoot was about to emerge was always placed up in order that all of the embryos might be equally exposed to the x-rays. The dishes were closely covered with three layers of moist Scott Tissue toweling to prevent evaporation from the seedlings during radiation. The controls were kept under the same conditions outside of the x-ray apparatus.

Immediately after treatment the seedlings were placed either in pots of damp earth for observation in the greenhouse or in jars of loosely packed sphagnum to be put into the incubators for root study. The irradiated plants grown in soil had the normal greenhouse conditions of light and temperature. A large number of pots were prepared at one time with the same lot of soil to insure uniformity of nutrition.

Results

EFFECT OF X-RAYS ON AERIAL PARTS OF *ZEA MAYS*

The following observations were made on a typical series raised in the greenhouse. Groups of 12 seedlings each received doses varying from 100 to 5000 r. Up to the fifth day there was no difference in the heights of the shoots of the different groups, but on the fourth day close inspection showed that there was a delay in the bursting of the sheath or coleoptile in the heavily irradiated plants. The number in each group with the shoots visible at this time was as follows: controls, 11; 100 r, 7; 200 r, 8; 1200 r, 1; 1600 r and above, 0. On the sixth day all of the controls, 100, and 200 r groups had

burst the sheath as had also about 50 per cent. of the plants of the more heavily irradiated groups.

Measurements made 8 days after irradiation showed that all growth of the sheath had stopped both in the control and in all of the treated groups. The following figures indicate that the net growth of the sheath was not retarded by irradiation: average height of control, 22 mm.; irradiated with 800 r, 25 mm.; and 2000 r, 23 mm.

From the sixth day, the growth in height of the plants receiving doses of 1200 r and higher fell behind that of the controls. The upper limit of the amount of radiation which allows the majority of seedlings to unfold the first leaf was about 1200 r. When a dose of 800 r was applied, the growth rate during the first month was equal to that of the controls when only the height of the shoots was considered. When part of this 800 r group was removed from the soil and measurements made 12 days after irradiation, the roots had an average length of 207 mm. as compared with 238 mm. for the controls. After three months the tops of the remainder of the 800 r group were about two-thirds of the height of the controls. This may mean that in the case of the shoots there was a very long latent period before the effects of irradiation could be observed. On the other hand, it is possible that this later dwarfing was caused by lack of root development owing to x-ray injury, rather than a direct effect of irradiation on the tops themselves. After a dose of 1200 r, most of the seedlings died in two weeks, having grown little more than the group which received 2000 r, while the survivors, a quarter of the whole number, grew as well as the plants in the 800 r groups. At a dose of 1200 r the individual sensitivity seemed most apparent. The individuals in the groups receiving doses between 1400 and 5000 r showed little variation, either in length of roots, which averaged 54 mm. at death, or in height of shoots, which averaged 45 mm. at that time.

The leaves of plants which received a dose of 200 r and more showed many small areas of yellowish green between patches of normal color. Up to 400 r, the first three leaves were the only ones showing this chlorotic disturbance. With higher doses, which still allowed fairly normal growth of tops, a much longer time elapsed before normally colored leaves were produced. With very high doses the occasional plants which were able to produce one leaf, were decidedly yellow with very small areas of normal green color.

Leaves in the 800 r group, which were of normal length, showed a peculiar twisting and curling in some cases. The edges of the leaf were slightly inrolled and tended to move toward the under side. This condition persisted throughout the three months of the experiment.

SPECIAL ROOT STUDIES

The seedlings grown in sphagnum were used for observations of the behavior of roots after irradiation. Tests showed that the plants could be

carefully removed from the jars twice a day without affecting the rate of growth. It can be seen from table I that the final length attained by roots

TABLE I

ROOT LENGTH ATTAINED IN SPHAGNUM AFTER KILLING DOSES OF X-RAYS

TEMPERATURE	DOSE IN R UNITS	HOURS AFTER TREATMENT	AV. LENGTH
°C.		hr.	mm.
22	10,000	77	32
,,	7,000	77	28
,,	5,000	77	31
,,	5,000	63	34
,,	4,000	63	31
,,	3,000	60	43
29	3,000	50	41
24	2,500	68	53
17–21	2,000	134	51
22	2,000	90	52
24	2,000	70	50
24	2,000	94	62
24	2,000	85	51
29	2,000	40	52
35	2,000	45	44
36	2,000	42	47
36	2,000	43	41

after receiving doses of 4000 to 10,000 r shows great uniformity. The growth curves are also practically identical throughout this range. A dose of 4000 r would then appear to be near the limit beyond which further irradiation could be expected to have no observable effect, at least under these experimental conditions. As the dose is reduced below 4000 r there is a fairly regular increase in the rate of growth of the primary root after irradiation. Doses below 150 r produce no measurable differences between such treated seedlings and their controls when primary roots alone are tested.

A study was made to determine the smallest dose that would consistently produce a measurable difference between such treated seedlings and their controls. Preliminary observations showed that the rootlets growing laterally from the primary roots are the parts of the plant which first exhibit visible evidence of x-ray injury, although these rootlets cannot be seen at the time of irradiation. It has been found (table III) that, in all experiments at a given temperature, the time required for lateral roots to appear on normal seedling is remarkably constant. Table II shows that the time between irradiation and the first appearance of lateral roots steadily increases with the dose from 75 to 1500 r, which is near the upper limit of the amount

TABLE II

TIME OF APPEARANCE AND POSITION OF LATERAL ROOTS WHEN GROWN AT 24° C. AFTER X-RAY TREATMENT

DOSE IN R UNITS	APPEARANCE IN AT LEAST 33% OF CASES			AV. LENGTH OF PRIMARY ROOT	AV. DISTANCE OF FIRST ROOTLET FROM SEED
I	TREATED	CONTROLS	DIFFERENCE	V	VI
	II	III	IV		
	hr.	hr.		mm.	mm.
60	61	61	0	94	0
75	84	68	16	116	0
150	93	68	25	103	0
300	100	68	32	113	.....
300	87	63	24	122	6
600	94	67	27	98	.....
600	96	67	29	110	13
1,000	90	64	26	90	.....
1,000	114	63	51	65	25
1,000	160	68	92	98	20
1,500	160	67	93	65	31
1,500	160	68	92	73	20

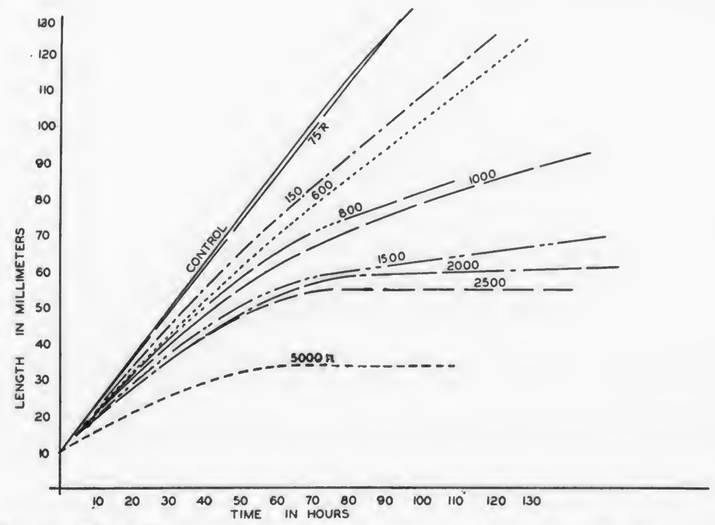

FIG. 1. Effect of various doses of x-ray on growth of primary roots of *Zea mays* in sphagnum at 24° C.

of irradiation that allows a few lateral roots to be formed on most of the seedlings.

Figure 1 shows that the growth rate of the primary roots treated with 75 r was identical with that of the controls, but the lateral roots in this irradiated group appeared 16 hours later than those of the controls. It has been stated above that 150 r was the smallest dose that could be depended upon to check the growth of the primary root in most of the corn seedlings.

Control seedlings grew at an increasing rate as the temperature was raised from 17° to 36° C. In these plants the lateral roots tended to emerge when the primary root was about 100 mm. long, irrespective of the time required to reach that length at various temperatures. Table III shows that

TABLE III

TIME OF APPEARANCE OF ROOTLETS ON CONTROLS AT DIFFERENT TEMPERATURES

TEMPERATURE	TIME OF APPEARANCE OF ROOTLETS		AV. LENGTH OF MAIN ROOTS	
°C.	hr.	hr. (mean)	mm.	mm. (mean)
37–39	39	39	112	112
36	38		106	
36	36	37	82	94
35	44		73	
35	40		129	
35	36	40	115	104
29	40		95	
29	36		91	
29	43		104	
29	39		108	
29	47		104	
29	45	42	96	98
24	63		90	
24	68		99	
24	67	66	119	103
22–24	96		122	
22	97	96.5	122	122

with increasing temperatures the time required for the appearance of lateral roots decreases, while the length of the primary root at this time is very constant. For instance, in a group raised at 22° C., after the usual germination period at 24° C., the lateral roots appeared 97 hours after the first measurement. In another group raised at 35° C. the rootlets appeared after 48 hours, the average length of the primary roots being the same in each case. Figure 2 shows the very regular rate of decrease in time necessary for the appearance of rootlets as the temperature increases.

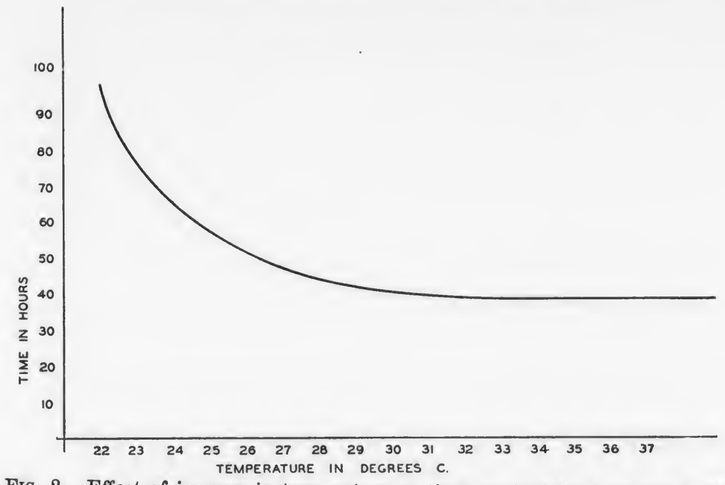

FIG. 2. Effect of increase in temperature on time required for appearance of secondary roots on seedlings of *Zea mays*.

Careful examination of the control seedlings showed that the rootlets first appeared on the primary root immediately below the grain in all cases, no matter how long their appearance was delayed by cold and the slow growth

FIG. 3. Section showing abnormal growth of secondary root after an x-ray dose of 1500 r.

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37–39	39	39	112	112
36	38		106	
36	36	37	82	94
35	44		73	
35	40		129	
35	36	40	115	104
29	40		95	
29	36		91	
29	43		104	
29	39		108	
29	47		104	
29	45	42	96	98
24	63		90	
24	68		99	
24	67	66	119	103
22–24	96		122	
22	97	96.5	122	122

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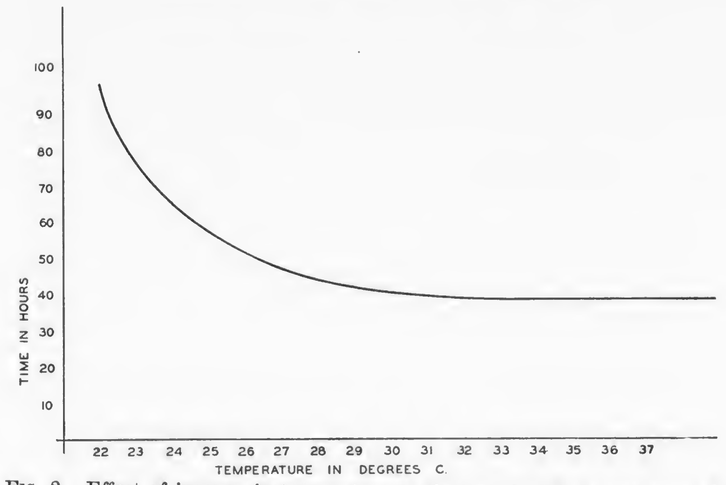

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FIG. 3. Section showing abnormal growth of secondary root after an x-ray dose of 1500 r.

INTENTIONAL SECOND EXPOSURE

accompanying it. It can be seen from column VI of table II that, when doses of 300 r and more were used, the average distance of the first lateral root from the grain increased from 6 mm. to 31 mm. after a dose of 1500 r. The roots grown in soil showed this same type of behavior after irradiation. With the higher doses the primary root had only a tassel of rootlets very close to its tip. Histological sections made from roots which had received a dose of 1500 r showed primordia of lateral roots in the region just above the level where rootlets were growing out in a normal manner. In some cases these primordial rootlets were subnormal in size and in a few examples they had turned either up or down, growing parallel to the longitudinal axis of the primary root, and they were entirely inclosed within its tissue. Figure 3 illustrates this condition.

Effect of temperature on growth after x-ray treatment

Experiments performed to test the effect produced by keeping the corn seedlings at 6° C. during irradiation with doses of 200 to 1000 r and then allowing them to grow at 24° C. showed no differences between such treated seedlings and those similarly irradiated at room temperature. Following the failure to produce differences in sensitivity to x-radiation by means of low temperature during treatment, a number of tests were made to find out whether varying the temperature after x-ray treatment would affect the amount of injury due to irradiation. Since corn was found to thrive at temperatures of 17° to 36° C., it was possible to observe the behavior of roots whose growth rates varied widely according to the temperature selected. In the first experiment three groups of seedlings were irradiated at the same time with 2000 r, and then the separate lots were placed, with their controls, in three incubators at varying temperatures. Care was taken to measure the growth at very frequent intervals. Figure 4 shows the curves made by the

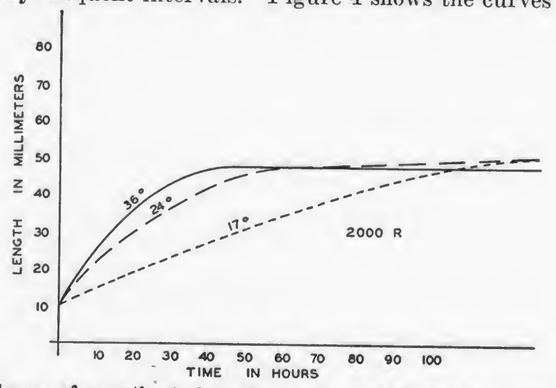

Fig. 4. Curves of growth at three temperatures after equal amounts of radiation. Length of life increases as temperature decreases.

growth of the primary roots of these seedlings exposed to 36°, 24°, and 17–21° C. after all were irradiated under exactly the same conditions. The shape of the curves is very significant, showing that the time of death was

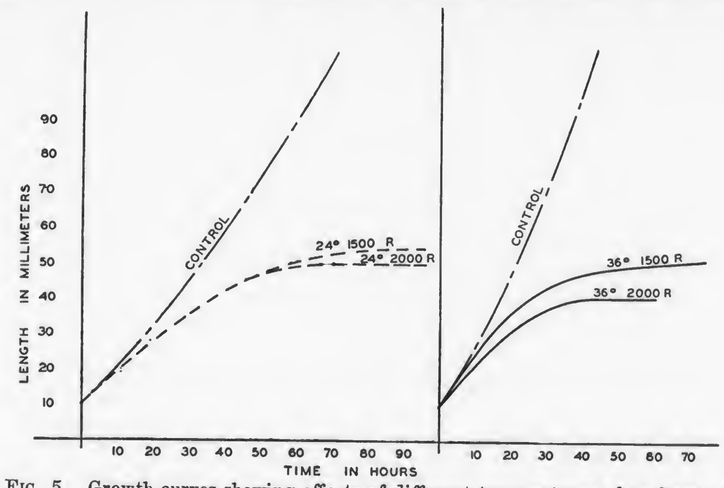

Fig. 5. Growth curves showing effects of different temperatures after doses of 1500 and 2000 r. Increasing doses decrease the latent period and the effect is exaggerated by increase in temperature.

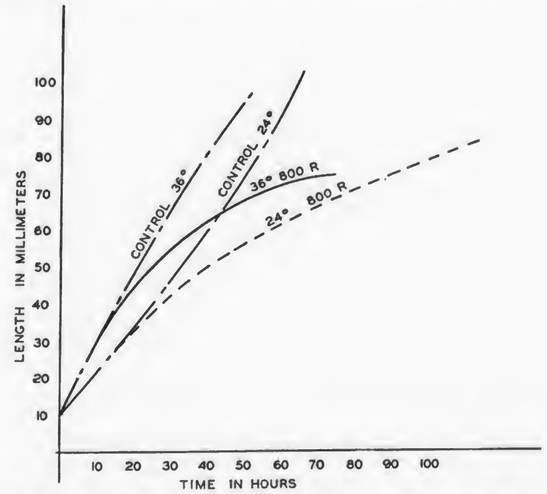

Fig. 6. Growth curves of primary roots of *Zea mays* showing effect of exposure to 24° and 36° C. after a dose of 800 r. At 20 hours the irradiated group at 24° C. shows no injury while at 36° C. roots are 4 mm. shorter than their controls.

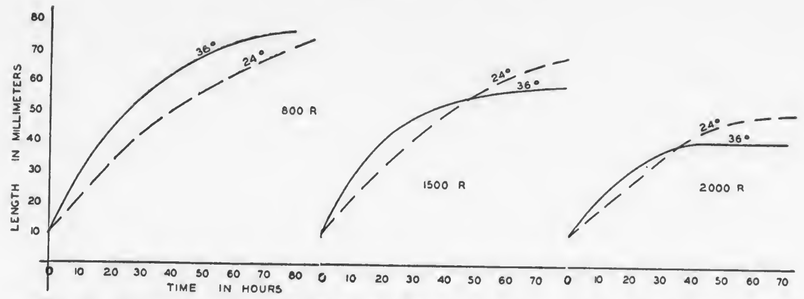

FIG. 7. Curves showing comparative effects of exposure to 24° and 36° C. after exposure to various doses of x-rays.

delayed as the temperature decreased. Similar experiments were performed by repeating the dose of 2000 r and also using 1500 and 800 r, in each case exposing part of the irradiated group to 24° and part to 36° C. with their respective controls at each temperature. Figure 5 shows that after both the 1500 and 2000 r treatments, the roots grown at 24° lived longer than those in the group at 36° C. Growth after 1500 r extends over a longer period of time at the lower temperature, and, also, the roots reach a considerably greater length before death when grown at 24° than at 36° C. Figure 6 shows that when the dose is lowered to 800 r, the irradiated groups grown at

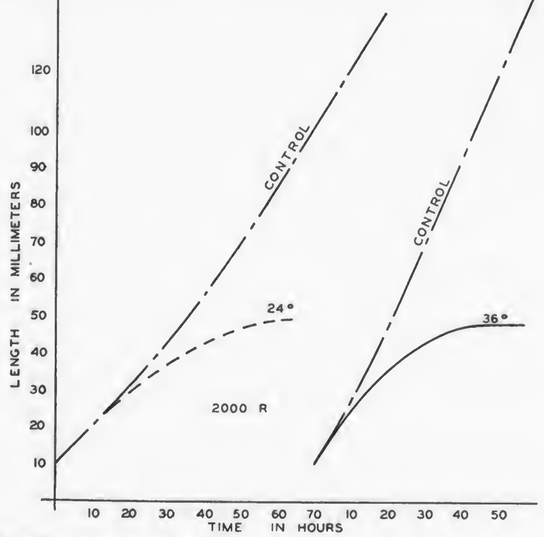

FIG. 8. Growth curves showing effect of temperature on length of latent period after an x-ray dose of 2000 r.

the two temperatures grow roughly parallel to each other, the 36° group growing the faster just as the 36° controls grow faster than the 24° controls. In figure 7 the curves of the growth following the three doses have been rearranged to show more graphically the effect of the two temperatures on each. The curves in figures 4, 5, 6, and 8 show that with all the doses used, the latent period was longer at the lower temperature. As the dose increases, the difference between the temperature effects is increased.

Discussion

The coleoptile was the only part of the corn seedling which failed to show any effects of irradiation. CATTELL (**3**) reported it to be the least sensitive part of wheat, as after a dose of 1200 r the coleoptiles showed a reduction of 8 per cent. in height while the primary roots were reduced to 45 per cent. of the length of the controls. It would appear that the coleoptile of corn is less sensitive to irradiation than the coleoptile of wheat. The apparent lack of sensitivity to x-rays of this organ may be partly explained by the results of cytological studies of *Zea mays* by TETLEY and PRIESTLEY (**18**). They found that cell division stops in the coleoptile earlier than in any other part of the embryo. The fact that cell division has probably ceased long before exposure to x-rays may account for this organ showing no injury after doses which seriously interfere with the growth of parts of the seedling in which there has been rapid cell division at the time of irradiation.

SKOOG (**13**), using moving pictures to study the behavior of the coleoptile in *Avena*, found that its growth stopped when it was ruptured by the shoot pushing through it. If it is natural for the growth to continue only as long as the sheath is intact, and if the rupture is delayed when the shoot is stunted by irradiation, then this tendency towards excess growth in height may mask the effect of x-ray treatment on the corn coleoptile. The fact that the coleoptiles of treated corn seedlings were a trifle longer than those of the controls may indicate that the stunting effect of x-rays was a bit less influential than the opposite tendency towards increased growth.

The chlorotic disturbance noted in the corn leaves is typical of the results reported for many species. NOGUCHI (**10**) examined sections of leaves from irradiated seedlings of *Helianthus annuus* and found that the light color was due both to a reduction in the number of chloroplasts and a deficiency in their chlorophyll content.

The fact that irradiation in any amount between 4000 to 10,000 r failed to produce more stunting of the primary root than did 4000 r, shows that corn responds to x-rays in this respect in very much the same manner as does wheat. FRANCIS (**5**) reported that doses increasing from 3390 to 13,000 r fail to produce any increase in damage to the seedlings either in affecting the growth or in reducing the rate of respiration. Many workers have tried in vain to find a dose of x-rays strong enough to kill the plant immediately.

In an early experiment, where it was necessary to compare the effects of irradiation on two groups of seedlings which had been treated under different conditions, the time for comparison was chosen when both groups had lateral roots starting but those of one set were further developed than those of the other. Quantitative measurements were made at first by carefully cutting off the rootlets, obtaining the dry weights of those of the two groups, and then computing the average weight of the lateral roots of each group. This laborious method was discarded when further study showed that small differences in the amount of damage caused by irradiation could be measured both by noting the time at which lateral roots appeared and also by recording the distance below the grain at which the first rootlets appeared. The use of the time factor is the more exact of the two methods. It is necessary to decide on a given proportion, such as one-third, of the plants to be required to show rootlets before the time of appearance is recorded. This helps to prevent mistakes being made when the group contains one or more freak plants which, for some unknown reason, are unusually resistant. Such individuals have been omitted in computing averages when they were few in number and extremely different from most of the group.

When preliminary experiments have been carried out at the same temperature and near the dose to be used, the time interval during which very frequent observations are necessary to establish the time of appearance of rootlets can be fairly accurately predicted. In order to compare the sensitivity of two groups of corn seedlings, a dose of 300 r would have the advantage of yielding data both on the time of appearance and the spacing of lateral roots below the grain, and it would furnish this information in a shorter time than would be required for the same observations after higher doses. WIGODER and PATTEN (**19**) observed that lateral roots of *Vicia faba* were delayed in the time of their appearance and were formed more and more distally from the seed as the dose of x-rays was increased. It is therefore possible that this type of reaction could be expected in many kinds of plants and could be used as a general method for comparing sensitivity to irradiation between groups of the same species.

The fact that the appearance of the first lateral roots is delayed by irradiation might indicate that time is required for the repair of certain damage before these roots can be formed in a normal way. The few sections which were prepared from seedlings which had been irradiated with 1500 r showed no indications of lateral roots being formed in the region just below the seed, while in the region further down there were abortive attempts at lateral root formation followed by a region producing apparently normal rootlets. Since irradiation is generally considered to have an aging effect on tissues, the failure of the lateral roots to reach the exterior along the upper part of the root might be due to the same type of conditions which prevent secondary

root formation in old, tough roots. Further study may determine whether the rootlets in the x-rayed material changed the direction of their growth because of the mechanical difficulty of penetrating the outer layers of the primary root.

In normal seedlings there was a quite definite relation between the length of the primary roots and the time of appearance of lateral roots, but this relationship was upset in the irradiated groups. Here the rootlets tended to appear while the primary root was relatively shorter, but if the plant's main root never reached a length of 60–70 mm. it was unlikely that any rootlets would be formed. It may be possible that as the plant begins to suffer from the slow growth of the x-rayed primary root, the lateral roots tend to appear earlier in proportion to the length of the main root as an attempt at compensation for the lack of absorbing power of the main root. JOHNSON (**7**) reported that irradiated seedlings of *Helianthus annuus* lack ability to absorb as much water as their controls. This condition is all the more serious for the plant, because the aerial parts, not being stunted by irradiation to the same extent as the roots, are in need of a supply of water approaching that of a normal plant.

There are reports of several experiments on the effect of irradiating living material at low temperatures. CRABTREE and CRAMER (**4**) treated mouse tumor with radium at 0° C. and found that its sensitivity to irradiation was greater than when it was treated at room temperature. MOTTRAM (**9**) obtained similar results, using *Vicia faba* (a very small number of plants) treated with both radium and x-rays. On the other hand, HENSHAW and FRANCIS (**6**) failed to find any difference in the sensitivity of wheat seedlings when irradiated at different temperatures. The same type of experiment was tried in this laboratory with corn, with the idea that this plant, being more sensitive to cold than wheat, might give somewhat different results. The findings were negative.

It was found that growing corn seedlings at different temperatures following irradiation produced differences in the length of the latent period, the ones grown at the higher temperature showing the damaging effects sooner than those which were allowed to grow more slowly at the lower temperature. This held true for groups of roots which had received a dose of 800 r and grew in a manner very nearly like the normal. The effect on the latent period became more and more noticeable as the dose was increased to 2000 r. It would be of interest to test higher doses to determine whether a stage could be reached where the damage done by irradiation would be so great that a comparatively low temperature could have no effect towards lessening the amount of injury.

With all biological material the time required for the effects of irradiation to appear varies according to the dosage, the type of organism, and probably

many other factors. It is generally considered that biological changes are the result of chemical changes set in motion by ionization during the actual time of irradiation. Conditions which would speed up or modify the chemical reactions could be expected to shorten the latent period required for the results of the biological changes to appear. Heat increases the speed of most chemical reactions and does appear to shorten the latent period in the experiments reported here.

Although irradiated seedlings grown at 36° C. begin to show the effects of irradiation at an early age, they grow fairly rapidly until they suddenly die. When groups of irradiated plants grown at successively lower temperatures are compared, it appears that the slower the plants grow, the longer they are likely to live. However, the dose of 2000 r has set a limit, perhaps within a range of 6 mm., to the amount of growth possible following that amount of irradiation. The seedlings grown at each temperature group die when their primary roots have reached this limit no matter how long a time is required for each group to grow roots of that length. This limit may be determined by any one of several factors or a combination of them. A given amount of food material may be available for the building of new root tissue and irradiation may prevent the formation or transportation of more of that substance after the original supply is exhausted. If the root is growing rapidly, it will use up its supply and die sooner than a slow-growing root which uses up its food more slowly. This theory might help explain why the primary root can grow to the same length, when either 4000 or 10,000 r have been applied. If there is the same amount of food material available originally for both groups of roots, the 4000 r dose is just as effective as the 10,000 r dose in preventing any further growth after the original supply is exhausted. As the dose was reduced below 4000 r this food formation was apparently less and less disturbed. Since growth of the plant as a whole proceeds quite normally after a dose of 800 r, it may be assumed that there is early recovery if this amount of irradiation interferes with the food supply.

Another cause of death may be the accumulation of mildly toxic substances until they interfere with natural physiologic processes, or, as SCHWARTZ (**12**) suggests, the proteins may be giving rise to increasingly toxic products as their decomposition proceeds. Either of these conditions would be aggravated and cause earlier death with increase in temperature and the higher rate of metabolism accompanying it. The difference between the length of the latent period at different temperatures in the case of plants receiving 800 r, might be due to the difference in the rate at which toxic materials were formed. It is possible that some of these poisonous substances may diffuse out; this would help explain part of the recovery which takes place when the doses of radiation are not too heavy.

The comparative lack of irradiation injury shown in the early stages by plants grown at 24° C. and below might be due to the time element of delay

made possible by the use of lower temperatures. Several authors have tested the effect of allowing time to elapse between irradiation and the beginning of growth processes following it. STRANGEWAYS and FELL (**15**) reported that when chick embryos were kept in the refrigerator for 24 hours after irradiation and then allowed to grow in incubators at their normal temperature, the injury due to irradiation was less than the injury suffered by embryos similarly irradiated but allowed to continue their normal growth rate immediately following exposure to x-rays. There may be a certain amount of recovery going on during the period before growth is resumed.

After living material has been irradiated there is a certain amount of residual effect which continues at a steadily decreasing rate. According to KINGERY (**8**), the rate of decrease of this effect with time follows a logarithmic curve. If growth processes are delayed for a time, the amount of residual effect will be growing less and less in the meantime. Therefore, when the rate of metabolism is allowed to return to normal there will be less damage done to the cells which are again beginning to divide than would occur if they continued dividing immediately after the irradiation period. In like manner, the long, slow growth made possible by low temperature could be assumed to reduce radiation injury by allowing more time for the reduction of the amount of residual radiation.

PACKARD (**11**) performed a set of experiments on Drosophila eggs which very nearly parallel the tests reported here on growing corn seedlings at different temperatures following irradiation. After the same dose of x-rays, groups of eggs which were incubated at 18° C. hatched in 80 per cent. of the cases, while in the group kept at 28° C. only 53 per cent. hatched. Low temperatures after irradiation also reduced the amount of injury shown by chick embryos in experiments reported by ANCEL and VINTEMBERGER (**1**).

The fact that corn seedlings, Drosophila eggs, and chick embryos all react to temperature effects after irradiation in the same general way would seem to indicate that temperature during this period controls one or more fundamental conditions involved in determining the amount of irradiation injury shown by the living organism.

Summary

Seedlings of field corn were x-rayed with doses of 60 to 10,000 r and their subsequent growth observed under varying conditions. The results are as follows:

1. The height of the coleoptile was not affected by doses up to 5000 r which was the highest dose used in these particular experiments.

2. Shoots of plants which received over 800 r showed the effects of irradiation by delay in bursting through the coleoptile and by failure to reach the height attained by their controls during the time of the experiment.

3. All of the greenhouse plants irradiated with 200 r and more showed a chlorotic disturbance in the early leaves.

4. Leaves of all plants which received 800 r, and the small number of survivors from the 1200 r group, showed a strong tendency to curl their edges toward the under surface.

5. The rootlets growing laterally from the primary root were the most sensitive part of the seedlings. Doses of 75 r and higher delayed the appearance of these secondary roots increasingly as the dose was raised, while doses of 300 r and higher increased the distance between the grain and the first of these roots. Comparison of the time of appearance and spacing of these secondary roots is suggested as a method for comparing the degree of sensitivity to x-rays of various groups of seedlings.

6. Doses up to 10,000 r failed to prevent at least 20 mm. of root growth after radiation was applied.

7. Plants irradiated with doses between 800 and 2000 r showed a much shorter latent period before x-ray effects were measurable when grown at 36° C. after treatment than when grown at 24° C. and lower temperatures.

8. After a dose of 800 r corn seedlings showed the same amount of injury whether they were grown at 24° or 36° C. After 1500 r the 24° group grew faster than the 36° group after 50 hours, and the roots attained considerably greater length during their longer life. After doses of 2000 r the 24° groups surpassed the 36° groups after 35 hours and lived longer, but after this dose the final length was never more than 9 mm. greater than that of the 36° group. On the whole, lower temperatures after irradiation tended to reduce the amount of radiation injury.

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METHODS OF RESEARCH ON THE PHYSICAL PROPERTIES OF PROTOPLASM[1]

WILLIAM SEIFRIZ
(WITH FIVE FIGURES)

Much excellent research has been done with a test tube and a Bunsen burner, but certain problems cannot be successfully attacked without the aid of intricate apparatus. It is the latter type of research, in so far as it applies to studies on the physical properties of protoplasm, with which this report deals.

Obviously, the first instrument with which the student of protoplasm must become familiar is the microscope. It had its beginning in the primitive use of lenses by the ancients. In the first book devoted exclusively to microscopical studies, "Micrographia," which was written by HOOKE in 1660, his compound microscope is illustrated (fig. 1).

It is not within the scope of this report to present a detailed description of the compound microscope. A knowledge of this instrument is extremely useful and it is suggested that the diagrams and descriptions of their optical systems appearing in the catalogues of leading microscope manufacturers be studied.

The microscope has a limit fixed, not by the magnifying power of lenses, but by the wave length of light and other factors which determine its *resolving power*. The highest powers of the microscope reveal objects which are about 0.1 μ (0.0001 millimeter) in size.[2] Greater magnification could be obtained, but it would be of no use, because it would then be impossible to distinguish between two particles or lines which are closer together than the length of the wave of light used. It is not greater magnification, but

[1] One of the purposes of this committee is to make available those methods of physical measurements which have proven useful and have passed their preliminary experimental stages. However, all methods should be regarded as steps in the progress of research which undoubtedly will be modified and improved from time to time as new facts are discovered. Obviously these reports cannot be exhaustive treatments. They are intended to point out fundamental principles and include a bibliography which should serve as a starting point for those interested in a more comprehensive study. In the present report of the physical methods committee, organized for the committee by Dr. SEIFRIZ, the immediate objective has been to discuss briefly the approved methods and apparatus used in determining the physical properties of protoplasm and cell structure.—EARL S. JOHNSTON, Chairman.

[2] μ = micron = 0.001 millimeter
mμ = millimicron = 0.001 micron
μμ = micromicron = 0.001 millimicron
Å = Ångstrom unit = 0.1 millimicron
μμ is often erroneously used for mμ.

IRREGULAR PAGINATION

A.J.S.

FIG. 1. The microscope of ROBERT HOOKE (1660).

greater resolving power that is needed. Resolving power is the capacity of a lens to separate one line or point from another lying very close to it. Let us take a specific case: A diffraction grating, as used for the formation of spectra, may contain 1000 lines to a millimeter. Microscope lenses of the highest power easily distinguish these as individual lines clearly separated from each other. But if there are two lines where there was but one before, then each line will be separated from its neighbor by 0.5 μ. It would, in this case, be barely possible to distinguish individual lines if the microscope lenses and the eye of the observer were of the best. We can go no further with direct vision. As a result, efforts to increase the powers of the microscope have of late been directed toward methods of illumination.

Illumination

Two lines of attack on the problem of microscopic illumination have been followed; either light of other wave lengths is used, or white light is applied in different ways. The light, other than composite white light, so far most successfully used is ultraviolet.

THE ULTRAVIOLET MICROSCOPE

The resolving power of a lens is proportional to its numerical aperture and inversely proportional to the wave length of the light used. In other

words, the greater the numerical aperture and the shorter the wave length of light, the smaller the structure which can be imaged by the lens. Written in the form of an equation:

$$\text{Smallest structure visible} = \frac{\text{wave length of light}}{\text{numerical aperture}}.$$

The average length of a wave of white light is 0.55 μ (5500 Å); accordingly, a lens with a numerical aperture of 0.65 will reveal an object $\frac{0.55}{0.65}$ μ or 0.85 μ in size. This means that if the lens is used to examine parallel lines 0.5 μ apart, the image produced would not contain separate lines, the structure would not be resolved, and higher magnification would be of no avail.

The resolving power of a lens increases with the angle at which light enters it, and with the refractive index of the medium intervening between the lens and the object viewed. The increase due to the angle of the entering light is proportional to the sine of ½ of this angle. If the maximum angle at which light can enter is 80°, the sine of ½ of 80°, or 40°, is 0.64. If the medium between lens and object is water with a refractive index of 1.33, the resolving power will be 33 per cent. greater than if it is air, the refractive index of which is 1.00; and if the medium is cedar-wood oil with a refractive index of 1.51, the resolving power will be 51 per cent. greater than in the case of air. ABBE, German optician, introduced the term "numerical aperture" (abbreviated N.A.) to include both of these factors. The numerical aperture is the product of the sine of ½ the maximum angle at which light enters the objective, and the index of refraction of the medium between the front lens of the object and the cover glass. To increase the resolving power of a lens, we can (as the above formula shows) decrease the wave length of light, or increase the numerical aperture. The latter has been done by lens manufacturers as far as practicable, with 1.6 as the upper limit. As the numerical aperture is increased, chromatic and spherical corrections are more difficult, and the working distance of the lens becomes less. We then turn to the possibility of using light of shorter wave length. Blue light is shorter than the average (yellow) light of the sun. It should, therefore, increase the resolving power of lenses. Ultraviolet light, having shorter wave lengths than the blue, should serve the purpose still better. For this reason ultraviolet has been used, and the method has given rise to the *ultraviolet microscope*.

By using ultraviolet light with a wave length of 275 mμ, as compared with 550 mμ, the average wave length of white light, it is possible practically to double the resolving power of a lens. The theoretical limit of the resolving power of an objective of numerical aperture 1.40, is 0.16 μ for a wave length of 450 mμ, and 0.13 μ for a wave length of 365 mμ. This theo-

retical value of 0.13 μ is only slightly above the lower limit of microscopic visibility. An objective of numerical aperture 1.40, with the 365 mμ ultraviolet spectral line (of mercury) will give 19 per cent. more resolving power than the best microscopic system with white light. But there are difficulties. Glass is practically opaque to light waves of less than 300 mμ, *i.e.*, to ultraviolet light; furthermore, such light is invisible to the human eye. The first difficulty is overcome by using quartz lenses, and the second by substituting the photographic plate for the human eye.

The ultraviolet microscope was presented to the biological world some thirty years ago by KÖHLER of the Zeiss scientific staff at Jena. The instrument has a potential resolving power twice that of the best optical systems using visible light, which means that it gives twice as much detail as do the best lenses with ordinary light. It also gives greater optical differentiation, *i.e.*, greater contrast, for example, between the translucent, glass-like parts of a cell. It thus obviates the need of staining protoplasm, which is difficult of accomplishment in living matter. The method was originally intended for metallographic studies, but in spite of a promising future, little outstanding work was done with the ultraviolet microscope. LUCAS (**14**), of the Bell Telephone Laboratories in New York, has revived its use.

Ultraviolet light of a known wave length is obtained by passing light from a suitable source, such as a spark between cadmium or magnesium electrodes, through a quartz prism which resolves or breaks it up into its component wave lengths. Ultraviolet rays of about 275 mμ wave lengths are selected. As these are invisible, the optical image cannot be seen in the ordinary way. It must either be photographed or visualized on a fluorescent screen. The fluorescent screen commonly used is made of uranium glass, and is mounted interchangeably with a camera just above the microscope. Magnifications of 5000 diameters result in sharp brilliant images with a degree of resolution surpassing by far that achieved with any other known optical system. With such an equipment, LUCAS has taken some excellent photographs of living cells.

Any wave length shorter than the average of white light will increase the resolving power of a lens. Consequently, light *just beyond* the visible limit of the violet end of the spectrum (the visible spectrum stops at about 390 mμ) should be better than white light, and it retains the advantage of white light in passing through glass. Ultraviolet of 365 mμ is, therefore, used with glass lenses.

The use of ultraviolet light for photographing living tissues is open to criticism because of the harmful effects which it may have on the cells. Certain microorganisms are destroyed almost instantly by ultraviolet light; others are mildly excited, and still others remain normal.

VERTICAL ILLUMINATION

Direct light, *i.e.*, rays coming from below the microscope stage, is the time-honored method of illuminating transparent material through the microscope. Obviously, opaque material cannot be so viewed, consequently it must be illuminated from above. The types of apparatus designed for illumination from above are numerous; the following are representative. The illuminator of W. A. Beck (fig. 2) is a simple and inexpensive optical

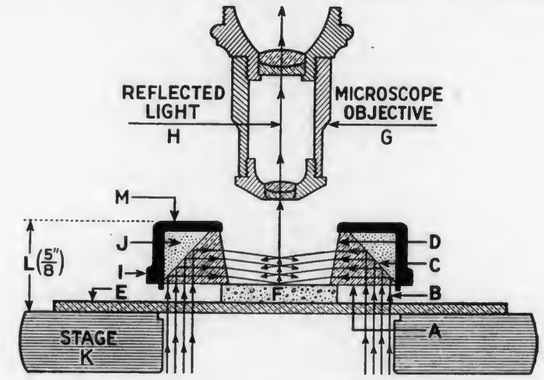

FIG. 2. The Beck illuminator. Light enters from below, is totally reflected by the prism C, and directed down upon the opaque material F.

system in which light comes from below, enters a circular glass prism from the upper silvered surface of which it is totally reflected to the opaque material lying in the center on the microscope stage. Similar, and as simple, is the "epi-mirror" of Carl Zeiss; it is part of the "epi-" equipment of Zeiss for dark-field illumination by incident light. One of the earliest of vertical illuminators is that invented by Silverman (manufactured by Spencer Lens Co.); instead of a reflector or prism, the light source is directly above the material surrounding the objective. Of a like design is the surface illuminator of Bausch and Lomb. It consists of a ring of electric lights surrounding the objective. Other more expensive devices for vertical illumination are the "Ultropak" of Leitz, and the "epi-condenser" of Zeiss. These latter instruments use light from a small and concentrated electric source placed close to the objective and entering it from above.

The vertical illuminator is a development of a metallurgical microscope which is used to advantage mostly in connection with the observation of highly-reflecting surfaces, such as those of polished and etched metals. Introduced by the metallurgist, the method has been adopted by the biologist (**32**) who finds it of advantage in studying not only opaque but also transparent material by viewing it in another light.

Dark-field illumination

When Faraday showed the Royal Society a suspension of colloidal gold, he told them that the solid particles of gold in suspension scattered light rays which strike them, so that a brilliant cone of light is produced. *Dark-field illumination* and the *ultra-microscope* are based upon this phenomenon. Colloidal matter is illuminated laterally against a dark background. Microscopically invisible particles thus become "visible" because of the light which they scatter. The direct beam of light from the source of illumination does not enter the eye of the observer; only the scattered rays from the particles are seen. The colloidal particles thus illuminated appear as brilliant spots against a black background. Two main types of lens systems are used to give lateral illumination—the cumbersome equipment known as the *ultra-microscope,* and the simple *cardioid condenser* or *dark-field illuminator.*

The development of the ultra-microscope is due to the Germans, Siedentopf and Zsigmondy. The instrument designed by them is a large and expensive apparatus which others have attempted to simplify. This type of ultra-microscope is sometimes referred to as the slit microscope, the slit being a small aperture corresponding to the hole in the shutter of a darkened room into which a beam of light enters and illuminates the motes in the air. The ultra-microscope consists further of a series of lenses and apertures, apart from the microscope proper, which direct a light ray into the colloidal material and thus illuminate it laterally. The Tyndall cone of a colloidal solution, as seen with the naked eye, is the total effect of the scattering of light by many particles, no individual particles being visible. In the ultra-microscope the individual particles are "seen" as centers of bursts of light. Thus viewed, a colloidal solution resembles the Milky Way at night, but with every "star" dancing about in active Brownian movement.

The simplest way to obtain a dark field with indirect illumination is shown in figure 3. Another simple method is the central stop diaphragm to be used with an Abbe condenser. It excludes all rays within the field of the objective's aperture. A dark field may also be produced quite easily by removing the top part of an Abbe condenser and substituting for it a dark-field element. The usual optical system for indirect illumination is the dark-field condenser. The same fundamental principles underlie this instrument as those of the slit type of ultra-microscope, namely, a black background, and indirect illumination. The light, however, enters the condenser from below as in an ordinary microscope.

There are several types and modifications, such as the *cardioid,* the *paraboloid,* and the *change-over* condenser. The first two names indicate the nature of the curve of the reflecting surface. The light rays strike and are

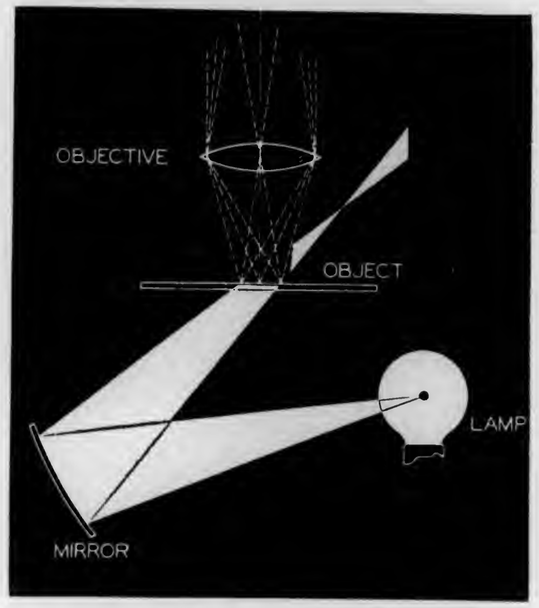

Fig. 3. A simple arrangement for dark-field illumination. (From Bausch and Lomb.)

reflected by two successive mirrored surfaces which direct and concentrate them at a point in the colloidal solution (fig. 4). Thus does the illuminating beam of light not enter the microscope, only the scattered rays from the colloidal particles being visible. The change-over condenser permits

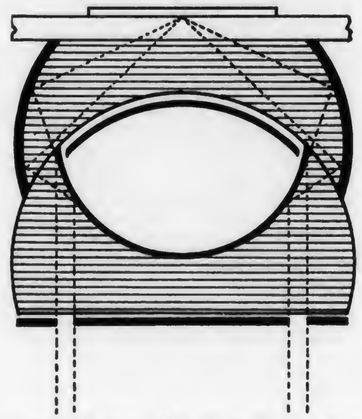

Fig. 4. The optical system of a cardioid dark-field condenser.

going from direct illumination, as in the ordinary microscope, to dark field without changing condensers.

A special dark-field condenser is manufactured by Bausch and Lomb which in itself takes the form of a moist chamber; it has been designed for work in microdissection.

The Spierer lens

The Spierer lens (**26, 28**), an ingenious development of dark-field illumination (o, fig. 5), is based on the principle that a colloidal particle scatters

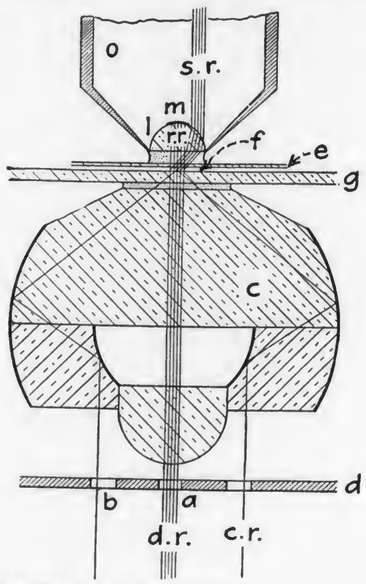

Fig. 5. The optical system of a Spierer lens:

o = objective
m = Spicrer mirror
l = lens
r.r. = reflected rays
s.r. = scattered rays
e = cover slip
f = material
g = slide
c = cardioid condenser
d = diaphragm
a = aperture for direct rays
d.r. = direct rays for Spierer lens
b = slot for cardioid condenser rays
c.r. = cardioid condenser rays

light unevenly, *i.e.*, there are more rays given off in one direction than in any other, namely, from that side which is away from the source of illumination. Degree of visibility of submicroscopic particles thus depends upon the angle of the illuminating ray. If a colloidal particle is viewed *toward* the source of illumination instead of at right angles to it, as in the slit ultra-microscope, or at 45 degrees to it, as in the dark-field (cardioid) condenser, the observer will then see the maximum amount of scattered light. A brighter picture, smaller particles, and a finer structure will consequently be discernible. In order to accomplish this, Spierer placed a tiny metal (silver, gold, platinum, or aluminum) mirror (m, fig. 5) within an oil-immersion objective. This metallic reflector covers but a small part of the lens surface. Light comes directly from below, and passes through the colloidal matter. It then enters the lens, where it strikes the mirror from which it is reflected downward, thus illuminating the particles a second time from above. The first illumination is, however, the important one for it directs the maximum brilliancy of the ellipse (or rather the three-dimensional ellipsoid) of scattered light into the lens. In the usual dark-field condenser a less bright portion of the ellipse enters the objective.

The presence of a mirror in the lens prevents direct light from entering but does not hinder the entrance of scattered light around it. In order that only so much direct light shall enter as can be reflected by the mirror, the aperture of the iris-diaphragm below the microscope stage must be as small as the mirror. The Spierer lens (o, fig. 5) is a dark-field system in itself; it may, therefore, be used with an ordinary Abbe condenser if the diaphragm is closed to a pinhole. But it is more convenient to use a special cardioid condenser (c, fig. 5) which has a fixed aperture (a, fig. 5) of correct size (*i.e.*, as small as the mirror, m, fig. 5). The use of a cardioid dark-field condenser adds still another ellipse of scattered light.

All dark-field methods of illumination depend upon diffraction phenomena for their usefulness. This is both an advantage and a disadvantage. The diffraction of light by ultra-microscopic structure makes that structure evident but the picture seen may not be an exact duplicate of the real one. It may be merely a diffraction image. This is not necessarily, nor always, true. One must, therefore, be particularly cautious in interpreting dark-field pictures. One can, however, always be certain that a diffraction image at least indicates that structure is there and usually it suggests the type of structure, if it is not an exact counterpart. With the aid of dark-field illumination, structural features in protoplasm not visible with direct light are made evident. Innumerable tiny particles in active Brownian movement are to be seen in protoplasm, as well as in the vacuoles of cells. Taylor (**30**) has determined the sign of the electrical charge on the ultra-microscopic particles in the protoplasm of a slime-mould; the particles were made visible by a cardioid condenser.

Ordinary light-field illumination usually shows the protoplasmic substratum to be homogeneous. Dark-field (SPIERER) illumination shows this apparently optically empty matrix to be a very fine emulsion consisting of two phases which have been termed *cryptoplasm* (hidden), *i.e.,* the optically empty background or matrix, and *phaneroplasm* (visible), *i.e.,* the emulsion globules. When once shown to be present by dark field, structures such as the fine protoplasmic emulsion can often be found with light field. SCARTH (**22**) has photographed the fine protoplasmic emulsion.

Tandem and inverted microscopes

Biologists often try new combinations of lenses, sometimes to the distraction of the physicist who fails to find any optical reason for the new combination. But the biologist finds it works better that way and so goes happily on; thus two microscopes may be used in tandem, the second picking up the image of the first. This permits the use of a low-power lens on the first microscope and therefore greater working distance between lens and object. Greater magnification is obtained through the second microscope, but the resolving power of the complete system is determined by the resolving power of the first objective.

E. LEITZ has designed, at the suggestion of CHAMBERS, an inverted microscope: the objective occupies a position under the cover glass, while the fluid drop lies upon it. This establishes conditions which greatly favor the technique of micromanipulation.

Vision at high speed

HARVEY and LOOMIS (**12**) devised a set-up which permits viewing a living cell while it is being centrifuged at high speed. The microscope, of course, is stationary. As the material passes under it, a light flashes on just for the moment so that one obtains an apparently continuous picture made up of many rapid successive exposures precisely as in the cinematograph. The gradual precipitation of granules due to centrifugal force can thus be observed while it is going on.

The polariscope

The physical (optical) properties of crystals differ in different directions. An object seen through certain crystals (*e.g.,* Iceland spar) appears to be double. This is known as *double refraction,* and is believed to be due to the *polarization* of light. Ordinary light vibrates in all planes extending out from the point of propagation, but the vibrations of polarized light are restricted to one plane. If a crystal polarizes light, it will allow light already polarized to pass through in one plane only, and two crystals will permit polarized light to pass through only if their planes of polarization

are parallel. If two such crystals are crossed, more or less of the polarized light will get through, depending on the angle of rotation. When the crossed crystals are at right angles to each other, no light can pass through. Such crossed crystals when built for the study of polarized light are known as *Nicol prisms;* mounted in an instrument they constitute a *polariscope.* The presence of double refraction and the polarization of light by crystalline matter may be detected and studied with the polariscope. The two prisms, the *polarizer* and the *analyzer,* are inserted, the one below the microscope objective (usually in conjunction with the condenser) and the other above the ocular, the crystalline substance being placed between them on the microscope stage. Rotating one of the prisms, which is mounted on a scale, gives in degrees the angle of the plane of vibration. If the material is not crystalline there will be no decrease in light intensity as the Nicol prism is rotated.

By means of the polariscope, chlorophyll within a living cell has been found to be crystalline, and as it is fluid, it must be of the nature of a liquid crystal. FREY (**10**) has ascertained the crystalline structure of cellulose in plant tissues by means of the polariscope, and BAILEY and KERR (**2**) have added to our knowledge of the lamellar structure of cell walls, with a series of excellent photographs taken through Nicol prisms.

It is of fundamental importance to consider the possible crystalline character of protoplasm. The polariscope has not shown protoplasm as such to be anisotropic, but it has shown striped muscle and contractile tissue in general to be so (*i.e.,* not having the same properties in all directions, as is true of crystals).

Microdissection

Microdissection and microinjection, with all forms of micromanipulation, are now included in the term *micrurgy.* The method, like all scientific discoveries, developed from primitive manipulative efforts on the part of pioneer workers. The first satisfactory instruments were constructed by BARBER (**3**) and by SCHOUTEN (**23**). BARBER built his pipette holder for the purpose of isolating single bacteria from cultures. The instrument is now replaced by several other types, each of which has its own peculiar advantages. The best known instruments which are commercially obtainable are the ZEISS-PÉTERFI (**21**) and the LEITZ-CHAMBERS (**6**) instruments. The TAYLOR (**31**) micromanipulator, privately manufactured, was an early design built for stability. A recent commercial model is that of FITZ (**8**), manufactured by Bausch and Lomb. A new but as yet little known model is that of DE FONBRUNE (**37**). It promises greatly to facilitate microdissection technique. It consists of a universal joint which controls three pistons operating against air. By means of three tubes, the pressure exerted by

the pistons is conveyed to a separate machine where three metal diaphragms, similar to those of aneroid barometers, are forced out or drawn in, and thus control, by means of levers, the rod to which the microneedle or pipette is clamped. The manipulator operates with remarkable precision, being wholly free from lost motion. The independence of operating and receiving mechanisms eliminates vibration. The hand of the operator and the needle-point move in the same direction and the operator's hand need never leave the one lever with which all movements are performed. DE FONBRUNE has also constructed an instrument for automatically drawing needles and pipettes under the microscope lens. In addition he has devised a simple method for protecting preparations of living material from dehydration without the use of a moist chamber. He simply places the material under an oil drop (nujol) either on the upper side of a slide or as a hanging drop. The oil is harmless, and lack of air has no ill effect for some time. Unfortunately the DE FONBRUNE micromanipulator is not yet commercially obtainable.

It is impossible to say which instrument is the best. The last mentioned gives promise of being so, but it has not yet been used by anyone except its inventor. Among the others, it is primarily a question of the instrument upon which one has learned. The LEITZ-CHAMBERS instrument is stable; the ZEISS-PÉTERFI has the greatest freedom of movement.

Accessories to micromanipulators are many. The fewer bought the better (certain ones sold are worthless). Exceptions are the needle-holders of Zeiss and the micropipette equipment of Leitz. Needles are best made by the individual investigator, because the type of needle should fit the work to be done, and the various types can be made only by hand. However, automatic devices such as drawing needles and pipettes are to be had (manufactured by E. Leitz). Microscalpel, microcauter, micromagnet, microelectrode, and microthermocouple are some of the appurtenances to micromanipulation. The construction of each requires a special technique.

The problems of micrurgy are many, and some have been quite successfully attacked. Certain of them are of interest not only in themselves, but have important bearings on related problems, thus the elastic quality of protoplasm, ascertained by micromanipulative methods, is of value as an indicator (the best we have) of the structure and fundamental nature of protoplasm. Other problems which remain untouched, or as yet unsolved by this method, are the structure of the chloroplast and of other plastids (is the chloroplast a droplet or a sac?), the removal of a chloroplast from one cell and its injection into another cell, the reality and nature of spindle fibers, the physical properties of the huge, motile, male gametes of cycads, and the eternal problem of the outer protoplasmic membrane and the tonoplast which is not yet fully elucidated to the satisfaction of all.

Electrical properties

The determination of electrical forces in organisms and cells is a fascinating study which is as yet in its infancy. These forces reach as high as a thousand volts, as, for example, in the ray-fish or electric eel, while that existing between the parts of plants, or between one cell and another, is of the order of 50 millivolts (0.050 volt). It is toward the measurement of these small electrical forces that our attention is here directed. This involves the use of microelectrodes (**15**) and a potentiometer circuit with amplifiers for greatest accuracy. Circuits for such measurements have been used and described by LUND (**15**) and his coworkers in the determination of potentials in trees.

Electrical investigations on cells and protoplasm require microelectrodes controlled by micromanipulative methods. Metal electrodes, even when of platinum, are likely to cause disturbances within the protoplasm; agar electrodes (agar with salt solution in a micropipette) are to be preferred. While in this review of methods we are concerned only with apparatus, it should be said that extensive work on other forms of potentials, namely, oxidation-reduction potentials, has been done with the aid of indicator dyes such as are used for pH determinations.

Cataphoresis studies have yielded some fundamental results on the electrical properties of living cells. The method involves subjecting suspensions of cells (or colloidal particles) to a difference in external potential; that is to say, the cells are placed in an electric field, and as all suspended particles, whether of metallic gold, oil, or living cells, possess an electric charge (with few exceptions), and, if free to move, they will migrate. The direction of migration is toward the pole of opposite sign to that of the charge on the particle. The rate is proportional to a number of factors including the potential of the external field and the potential (the interfacial potential) of the particles. These factors are all taken care of in the HELMHOLTZ formula for cataphoretic migration (**37**). With as yet no convincing evidence to the contrary, it can be definitely stated that all living cells are negatively charged when suspended in a solution similar (in regard to salt concentration and pH) to that in which the cells normally live. The interfacial potential of colloidal particles and cells in suspension is of the order of 30 to 50 millivolts; settling out (for example, of bacteria) occurs at about 11 millivolts.

The apparatus so far most extensively used in America for cataphoretic work is the NORTHROP-KUNITZ (**20**) model. It consists of a very shallow glass chamber open at the two ends to receive the electrolytic solution which connects the chamber with two poles of zinc immersed in zinc sulphate. A more recent model is that of ABRAMSON (**1**) which is less expensive and has the advantage of rigidity as it is constructed of one piece. The

NORTHROP-KUNITZ model is commercially obtainable. The ABRAMSON model is as yet only privately made.

To make simple and relatively crude cataphoretic observations is an easy task, but to make accurate measurements is quite another matter. The beginner is cautioned against the use of simple types of cataphoretic chambers which have been offered for sale. They are well-nigh useless and the results obtained with them are wholly unreliable. Practically all work purporting to show that cells are positively charged is due to the use of such, or home-made apparatus. Failure to measure pH is sometimes responsible for the trouble, for the sign of the charge on many suspended particles can be reversed by a change in the pH of the solution.

Simple comparative and reasonably accurate experiments in cataphoresis can be performed without calibrating the instrument, but for exact work this must be done. The best method for doing so is that of ABRAMSON (**1**).

Work in cataphoresis has yielded some of the most interesting and possibly far-reaching results of recent experimentation on the physical properties of cells. Only brief reference will be made to this work here, merely to call the attention of the student to articles wherein technique as well as results are described. Some of the earliest research on the cataphoretic migration of colloidal particles and bacteria was done by FREUNDLICH (**9**); then followed work by NORTHROP and KUNITZ (**20**) establishing the *critical* potential of bacteria. FALK (**7**) correlated the pathogenicity of bacteria with their potential. (This work may prove in part erroneous, but it was highly suggestive, pointed the way, and may yet be right to a degree.) More recent is the important work of MUDD (**17**) on sperms and bacteria, of ABRAMSON (**1**) on protein-covered quartz particles, and of MOYER (**16**) on latex. The last-mentioned investigation has made a fundamental contribution by showing that kinship or species relationship of Euphorbias is related to the shape of the mobility curves, and, primarily, to the iso-electric points of the latex particles.

The centrifuge

The centrifuge has played a part in studies on protoplasm chiefly in regard to determinations of viscosity. The first work of this kind was done by NĚMEC (**18**). He found that changes in the viscosity of protoplasm occur during cell division. In the young dividing cells of the onion, bean, etc., resting nuclei are, by centrifuging, thrown against the apocentric cell wall, while spiremes and asters remain situated in the center of the cell. Metakinetic and telophasic figures are only slightly displaced. The most easily disturbed stages of cell division are those coincident with the formation of the cell wall. In other words, the protoplasm of the resting cell

preparatory to division is of low consistency; the protoplasm of the cell in mid-mitosis and immediately following is of high consistency, and the protoplasm of the cell in the last stages of division is of minimum consistency.

Recent developments have resulted in the construction of a small air-driven centrifuge which attains the highest speed yet known for centrifuges (**5**).

Centrifuging is but one of several ways of determining protoplasmic consistency. Other methods are microdissection (**24**), magnetic properties (**37**), the fall of statoliths (**37**), plasmolysis (**34**), and Brownian movement (**4**). Misunderstandings which have arisen, and a convincing clarification of them, have recently been set forth by FRY (**11**); he employed the centrifuge method.

All determinations of protoplasmic consistency by whatever method involve a consideration of the important question of the applicability of the single constant laws of Newton, Poiseuille, *et al.*, developed for true solutions and pure liquids, to systems such as protoplasm, the behavior of which is anomalous. The problem is an important one and involves recent rheological theories on non-Newtonian fluids (**37**). Investigations on the viscosity of protoplasm are many and the bibliography extensive; these have been reviewed by WEBER (**33**) and others (**25**).

Photography

Photographs supply an authentic record of an experimental result. Photomicrography is now so highly developed a science and art that a discussion of it here would be wholly inadequate. A few remarks of interest and perhaps value may, however, be made. The small, $4\frac{1}{2} \times 6$ or 9×12 cm. camera which rests directly upon the microscope tube is very convenient, and excellent results can be obtained; but many workers still prefer to use the old-fashioned camera-box swung upon an upright support. The latter equipment is certainly the more flexible of the two, but it lacks one very convenient attachment of the former—the beam-splitter. It is a decided advantage to be able to focus through an ocular rather than upon a ground glass. Certain new but expensive photographic instruments combine the advantages of the old type camera-box and the new microscope camera with beam-splitter. The production of good photomicrographs is to a considerable degree dependent upon a correct choice of light filters. These can be had in considerable variety. The infrared filter is a recent addition and is to be used with special infrared sensitive plates. The photographer must try out the various combinations of lights, filters, and negatives suitable for his particular material.

The introduction of cinematography into microscopic work has yielded some fascinating results which are of real value to the research worker as

well as to the teacher or student. With the aid of moving pictures, details in mitosis heretofore unsuspected have been discovered. (A word of warning should be given in regard to the misleading impression created by speeding up films; pictures taken at the rate of one a second or one a minute then shown at the rate of 32 a second often give a highly entertaining but scientifically inexact impressions.) Motion pictures of tissue-cultures show the migration and division of fibroblasts and the movement of chromosomes in culture; others illustrate the development of the growing mammalian embryo from the one-celled stage to the sixteen-celled stage. Very striking are moving pictures taken with dark field.

For botanists, moving pictures have made their chief contribution in such studies as those on the mechanism of conjugation in *Spirogyra*, and on the operation of the animal-trap of *Utricularia*. These studies have been carried out by LLOYD (**13**). Through them is the method of movement of the male gamete in *Spirogyra* for the first time fully understood (motion is by contraction of vacuoles). A recent application of the cinematograph to protoplasmic studies has involved photographing protoplasmic streaming. Streaming protoplasm is always fascinating to watch, and becomes even more so when seen on the screen. But the method has a practical application; events which take place but once and too hurriedly to permit careful observation can be seen again and again if they have been photographed as moving pictures and are then shown at slow speed. The equipment necessary for taking moving pictures can be expensive but need not be. An essential feature is the beam-splitter so that material may be seen while it is being photographed. Other than this, any type of amateur camera for cinematography is all that is needed. The 8-mm. film is fairly satisfactory, but better results are to be had with the 16-mm. or professional 32-mm. film. The saving in cost of the 16-mm. over that of the 32-mm. film is to be considered in regard to the film, camera, and projector. Perhaps in no other way can an idea of the perpetual activity of protoplasm be so dramatically conveyed as through a moving picture of the streaming protoplasm of myxomycetes.

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THAT WORD "EMULSOID"

SHAKESPEARE wrote, "What's in a name?"; but then, he had never heard of "emulsoids." No word has caused more confusion in the colloid-chemical thinking of physiologists than "emulsoid." Biologists continue to take it at its etymological value, while most chemists have long ceased to regard it in this, its original sense. The term was coined by Wolfgang Ostwald in the early years of the present century, when colloid chemistry was still in its infancy, as sciences go. Originally "emulsoids" included coagula, jellies and emulsions, the term being based on the assumption that jellies are fine emulsions. Ostwald's only evidence for assuming that jellies are "emulsion-like" was that both they and emulsions increase in viscosity with increase in concentration of the dispersed phase, while "suspensoids," or solid colloidal suspensions, do not do so. The evidence on which the term was based is correct, but it tells a very small part of the whole story and is entirely misleading. To this day, the concept that jellies are fine emulsions clings, in spite of repeated and substantial proof to the contrary.

My own interest in this matter rests on the mischievous influence of the word "emulsoid" on interpretations of protoplasmic structure. All agree that protoplasm is an "emulsoid," but those biologists who know of the word only from the reading of old and standard texts conclude that protoplasm must be a liquid-liquid system, for that is what the word "emulsoid" means, *i.e.*, "emulsion-like," and emulsions are liquid-liquid systems. Other workers, knowing the historical background of colloid chemistry, realize that the word "emulsoid" has long since lost its original meaning, for no chemist well grounded in his knowledge of the colloidal state believes jellies to be like emulsions, except in that one property which Ostwald selected for the basis of his classification. In every other respect, dispersions of gel-forming substances are quite distinct from emulsions. Whatever their structure, jellies are not liquid-liquid systems.

Though there are some interesting experiments which indicate that protoplasm behaves like an emulsion, many physiologists regard the similarity as purely incidental and superficial. The emulsion

hypothesis of protoplasmic structure satisfies but few conditions, and is wholly contrary to many of the most significant properties of living matter. Among speculations on the emulsion nature of protoplasm, there is one that has recently come to my attention, namely, that the human brain is an emulsion; not only this, but it is said to reverse from oil-in-water to water-in-oil. While I know little about the histology of the brain, I am convinced that the hypothesis is based on the keen sense of humor of its author, for a brain that is an emulsion of water dispersed in a continuous phase of oil is a brain through which little can penetrate. Perhaps the author of the theory has some colleague in mind!—The brain consists of cells, or so-called cell-bodies, with their fibrous axons and dendrites, interwoven by a supporting framework of neuroglia, the whole bathed in fluid: this is not an emulsion.

It is not, however, my purpose here to discuss the "emulsoid" hypothesis of protoplasmic structure, but rather to plead with physiologists to give up ideas on gel structure which most chemists have long since discarded.

The controversy is an old one, but I had rather assumed that it was amicably settled, yet on reviewing of late a number of text-books on physiology and chemistry, the persistence of the theory was brought to my attention in a striking way. A note, therefore, seems worthwhile.

None of us treads with security when wandering far from our chosen field of research, as every physiologist must if he covers his subject in a broad way, but an author may save himself and his readers from old and discarded ideas by simply turning to an authoritative source. In the present instance, the colloid chemist is the man to whom physiologists must turn. An outstanding authority such as Freundlich[1] says that the word "emulsoid" must either be dropped or its original and etymological meaning ignored.

Selections from five new books, four in physiology and one in chemistry, illustrate how poorly biologists and some others have heeded this advice of Freundlich.

In one text, emulsoids are defined as liquid-liquid systems, and milk cited as an example. On the next page, the author states that in addition to "these various types of colloidal suspensions, mention should be made of emulsions," which he again defines as liquid-

[1] "Capillary Chemistry," 1926.

liquid systems with milk as an example. What is the student to make out of this, for not only is an emulsoid given the twenty-five-year-old and discarded definition of Ostwald, but the author tells the student that *another* type of system will now be considered, namely, emulsions, and then gives the same definition and the same example already stated for emulsoids.

In the second text, "colloidal theories" are introduced by a reference to "emulsoids," then, without any break in thought, the emulsion hypothesis of cell permeability is presented.

Two other text-books publish the diagrams first used by Bayliss and intended to illustrate a hydrosol and a hydrogel. The first figure is of black spots on a white background and labelled "hydrosol"; the second is of white spots on a black background and labelled "hydrogel." These are typical diagrams of an oil-in-water and a water-in-oil emulsion.

Bayliss refers to the scattered black spots of the "hydrosol" and the black background of the "hydrogel" as solid matter and thus regards the hydrogel of gelatine as similar to that of silica. This is in keeping with the micellar theory of gel structure which Bachmann thought he had shown to be true for gelatine. The error of Bayliss lay not so much in supporting the micellar hypothesis of gel structure but in assuming that water is firmly held in a jelly because it is "imprisoned" between "solid walls."

Water is firmly retained in jellies not because it is trapped in pockets or in isolated drops, but because it is bound by adsorptive or other forces.

Those who have copied the black and white diagram of Bayliss misinterpret the situation still more by referring to jellies as fine emulsions and to the setting of gelatine as involving phase-reversal. The figure is labelled "a diagram to illustrate the reversal of phases during sol-gel transformation." With this statement the assumption is again made that "enormous pressure is required to squeeze water out of a set hydrogel," because "the previously continuous phase—becomes the dispersed phase."

The author of a recent book on the colloidal state of matter asserts that liquid-in-liquid systems *include* the "emulsoids," and these in turn "contain the emulsions." The author realizes that emulsions are not good "emulsoids" and suggests that they be put in a separate group, but he gives as reasons that many emulsions are outside of the colloidal range because of

the size of the dispersed drops, and many are not simple two-phase systems; all of which is true, but these are not the reasons why emulsions are not "emulsoids," as the term is now generally used.

The structure of jellies is not known with certainty, though much progress on this subject has been made in the past quarter of a century. At the first Colloid Symposium (in 1923) a preliminary (and as yet unpublished) report on the structure of gelatine was given. The speaker referred to the gelatine molecule as a fiber of great but definite length, with a cross-section of not more than a few Ångstrom units. It is only necessary to postulate an interweaving or other specific grouping of these molecular fibers to obtain the type of structure now very generally applied to jellies and to protoplasm.

Whether the fibrillar (chain molecule) or micellar theory of gel structure is the correct one is unimportant so far as the point under discussion is concerned. Here we are concerned only with the generally held point of view that jellies are not fine emulsions and gelatinization does not consist in a reversal of phases.

Ellis[2] and Donnan stated in 1913 that neutral oil emulsions are model *suspension* colloids; that is to say, they are "suspensoids," and not "emulsoids." Four years later, Hatschek,[3] on the basis of a neat mathematical-physical analysis, asserted that "the theory that gels consist of two liquid phases must be pronounced untenable." McBain[4] has since found that there is no change in the electrical conductivity of a soap sol when it sets to a gel, which means that the continuous and conducting phase remains the same; there is, therefore, no reversal of phases. The work of the x-ray investigators, Sponsler,[5] Astbury,[6] *et al.*, should be sufficient to convince one that gels and jellies are not emulsions.

Much confusion and erroneous instruction will be avoided if the word "emulsoid" is discarded. It was based on a misconception and is no longer used by well-informed chemists in its original sense.

So with Lady Macbeth, I cry, "Out, damned *emulsoidal* spot! out, I say!"

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[2] Transactions of the Faraday Society, 9: 14, 1913.
[3] Transactions of the Faraday Society, 12: 17, 1917.
[4] *Jour. Phys. Chem.*, 28: 706, 1924.
[5] *Jour. Gen. Physiol.*, 9: 221, 1925.
[6] "Fundamentals of Fibre Structure," Oxford, 1933.

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A THEORY OF PROTOPLASMIC STREAMING

PROTOPLASMIC movement is now recognized as a general and fundamental property of metabolically active cells. It has risen to this position from the controversial one held not many years ago when the streaming of protoplasm was looked upon by some physiologists as a pathological condition in opposition to the view that it is evidence of a normal and healthy state.

The force responsible for protoplasmic flow has been the subject of much lively and stimulating speculation. My earliest memory goes back to the days when surface tension, the *sine qua non* of so many cellular activities, was regarded as the energy source of protoplasmic streaming. There was also the suggestion that the one-way "shuttle" type of flow in the filaments of coenocytes, where movement is first in one direction and then in the other, may be due to hydration at one end and dehydration at the other end; but the protoplasm flows equally well in both directions, even when fully submerged. Obviously, dehydration can play no part.

Physiology next entered the colloidal epoch when it became apparent that many cellular activities had their counterpart in colloidal systems. There thus arose the suggestion that protoplasmic streaming is a cataphoretic migration of particles or the electro-endosmotic flow of an aqueous medium; if either, it is the latter, for in streaming protoplasm the entire mass of material moves and not just the suspended particles. In spite of some attempts to prove that streaming protoplasm is associated with electric potentials, there has been no convincing evidence that the

potentials measured are real, in the sense of innate to the protoplasm, and if real, are the cause rather than the result of the streaming. Most electrical determinations made on cells and tissues are subject to the criticism that the potentials measured may not reside in the living material alone, but are produced by the experimental equipment as a whole. This statement is in no way intended to deny or question the existence of electric potentials in organisms; on the contrary, all necessary conditions for the production of potentials are present, but do the potentials measured exist in the living material?

Should the source of energy responsible for protoplasmic streaming be found, there would still remain the question, why the direction of flow reverses in the shuttle type of streaming. The protoplasm of slime-molds flows but one way at a time. Were a potential responsible there would have to be a reversal of it every forty or fifty seconds, and so the problem takes on a two-fold aspect—why the streaming, and why the reversal?

The slime-molds, or myxomycetes, sometimes referred to as mycetozoa, possess a body or plasmodium which is a non-cellular protoplasmic mass with ever-shifting channels of flow. A brief study of slime-mold plasmodia is sufficient to suggest the presence of a nearly perfect rhythm in the reversal of direction of flow. The average of many time records gives forty to fifty seconds for each period of flow. The minimum time is twenty seconds, and the maximum sixty between reversals. (The average maximum rate of flow is 0.07 mm per second.) The foregoing limits in time of flow suggest a lack of rhythm, but most often the periods of flow vary little from the mean. Pronounced divergences from the average of forty-five seconds are to be attributed in part to physiological disturbances in an artificially mounted specimen of plasmodium.

My own motion pictures of slime molds had all been taken at moderately high magnification and at normal speed. I therefore failed to see the essential feature of the rhythm. This first became obvious to me on viewing the excellent cinematographs of slime-molds taken by Drs. J. Comandon and P. de Fonbrune, of the Pasteur Institute at Garches, France. My visits to these laboratories have always been profitable. The first films of slime-mold plasmodia by Dr. J. Comandon and Professor P. E. Pinoy were made as long ago as 1912.

When the moving-picture of a small plasmodium is taken at one hundredth of the normal speed and projected at the customary rate, the entire plasmodium is seen to go through rhythmical contractions and expansion similar to the pulsation of a heart. At each contraction, the direction of flow reverses. The mechanism of protoplasmic movement in slime-molds is, then, one of rhythmical contraction and relaxation of the plasmodium as a whole, with a period of 40 or 45 seconds for each pulsation.

If the area covered by a plasmodium, or a branch of it, is noted with the aid of a micrometer, it will be seen that the plasmodium contracts with outgoing protoplasm and expands with incoming protoplasm. I had interpreted this swelling and shrinking as the result instead of the cause of streaming. Contraction is not due to the exit of fluid, and expansion to its entrance, but on the contrary, as in the case of the heart, the exit and entrance of fluid are due to the contraction and expansion of the living substance.

The beat of the heart is controlled by a sympathetic nervous system. There is no nervous system in the sense of nerve-centers and nerve-fibers in slime-molds, but these are not necessary in order to have response and control.

Some modern workers are inclined to strip protoplasm of all the attributes of higher organisms, for-

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getting that many of these attributes exist because they are properties of protoplasm. The tendency to regard the protoplasm of more primitive forms of life as less intricate, less responsive, if not "less living" than that in more highly developed forms is evident in such statements as, "there is considerable objection to the use of the word 'injure' in reference to plants." I recall the delightfully courteous remark of the English chemist, H. R. Proctor, who, finding my mechanistic interpretations of protoplasmic behavior rather harsh, asked if he might not still be allowed to regard life as, so to speak, a new departure.

In my turn, I ask the reader merely to admit that protoplasm is alive, for in so doing he tacitly grants that it exhibits irritability, in other words, nervous response.

It is interesting to contemplate the possible relationship between the rhythmical pulsations responsible for protoplasmic streaming in myxomycetes and the rhythmical contraction of sympathetically controlled muscle-tissue.

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FRANÇOIS ANDRÉ MICHAUX, THE BOTANIST AND EXPLORER

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(*Read April 24, 1937*)

ABSTRACT

This paper sketches in outline the chief events in the life of André Michaux and that of his son, the subject of the sketch. The two are considered because their work was so very thoroughly intertwined.

The chief botanical explorations of François André Michaux are outlined, and something of the botanical accomplishment of both the father and the son stated.

The relation of the son to the American Philosophical Society is pointed out, and the story of the Michaux Memorial Grove in Fairmount Park has been brought up to date.

THE person who finds himself called upon to write on Michaux, the botanist and explorer, may well find himself perplexed as to the course to be pursued. There were two Michauxs, both eminent in our early history for their botanical accomplishment and for their wide-ranging explorations. Father and son, they have left records of achievement that will make the name ever illustrious in the annals of American botany.

André Michaux, the father, is the man who comes first to the mind of the botanist. His name was rendered memorable by his discovery and description of a host of plants that had not been brought to the attention of science before his time. By virtue of close observation and by his clear descriptions of the new plants found, he is now credited with the discovery of over three hundred species of plants.

I have looked through the 7th edition of Asa Gray's Manual of Botany for plants credited to Michaux, senior, in an area bounded on the north by the 48th parallel from the Gulf of St. Lawrence to Lake Superior and the international

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boundary, thence to the northwest corner of Minnesota; on the west by the western boundary of Minnesota and northwestern Iowa, then southward along the 96th meridian; and on the south by the southern boundaries of Kansas, Missouri, Kentucky and Virginia. This area was but part of the country traversed by him. He traveled from Hudson Bay to Florida, along the eastern side of the continent, and penetrated westward to the Mississippi River.

Accredited to André Michaux in that portion of the country seen by him and included in the narrower range of the Manual, I find 24 new genera of flowering plants. He discovered and first described 293 species of flowering plants, of which 91 have since been referred to genera other than those to which Michaux assigned them, leaving 202 species unchanged in the present botanical record, in spite of over a century of the most critical study. The persistence of the name of Michaux in textbooks now in daily use is due almost entirely to the explorations, critical study and accurate description of material collected by the father, André Michaux.

His son, François André Michaux, however, is the main subject of my sketch, although their lives and work were closely interwoven until the death of the father.

André Michaux, like his son, François André Michaux, was born on a royal estate at Satory, near Versailles, France. The Michauxs for generations had been in charge of this estate. André, *père*, with whom our interest begins, was born here in 1746, and grew up on the estate under his father's instruction. His marriage in 1769 to Cecil Claye, the daughter of a wealthy farmer, was followed after eleven months by her death, leaving André with the infant son. It is said that as a relief to despondency he turned to an intensive study of botany under the eminent French botanist, Bernard de Jussieu. This brought him nearer to the Royal Gardens of Paris. He soon began actively to collect plants, visiting England, Auvergne and the Pyrenees. A diplomatic appointment took him to Persia and Mesopotamia, where he collected

seeds and plants for introduction into France. After three years in the Near East (1782 to 1785), André returned to France, and was immediately sent by the Government to North America, then little known to science, to study the forest trees, with the object in view of introducing into France such species as might prove useful in naval and other construction work.

On October 1, 1785, André Michaux, his fifteen-year-old son, François André, and a practical gardener, Paul Saulnier, landed in New York, and proceeded to establish a nursery near Hackensack, New Jersey, in which American trees were to be grown for shipment to France. The interest in applied science at that time centered in Rambouillet, and to the Rambouillet nurseries André Michaux's trees, to the number of 60,000, were sent. After a year and a half spent in botanical explorations and in establishing the northern nursery near New York, father and son went to Charleston, South Carolina, leaving Saulnier in charge of the first nursery. The year 1787 saw the purchase of 220 acres of land about ten miles from Charleston, on which a second nursery was to be developed, and on which trees of more southern origin were to be grown for shipment from the port of Charleston.

In the year 1787, exploring expeditions were made into Florida and to the Bahamas, also a strenuous excursion up the Savannah River to the head waters, over the Appalachians into the Tennessee Valley and then the return to Charleston. A visit was paid to the northern nursery in that busy year.

The son was retained at Charleston, probably much of the summer, either because of ill health or to manage the new nursery in his father's absence. His troubles were increased in 1789 when he was shot by a companion hunter, the projectile injuring his left eye. This accident seems to have been much above the horizon during the year and longer.

Troubles at home in France began to interfere with plans in America. Remittances ceased, the faraway scientists were out of sight, and much trouble was near at hand. Michaux,

père, advanced what money he could to continue the work, and pledged his property in France to secure loans.

Early in 1790, François, by this time a grown-up young man of twenty years, returned to France, now in a state of revolution. He espoused the popular cause, although the family had for generations been closely attached to the Royal interests, and his own childhood had been spent on a part of the Royal domain. Perhaps his course of action was somewhat influenced by what he saw in the nurseries at Rambouillet. He found there but a few of the trees that his father had striven so earnestly to secure for France. Half the total number had been given by the Queen to her imperial father in Austria, others had been given to members of the Court to embellish their private estates, and those that remained in the Royal Nurseries had been shamefully neglected.

François seems to have spent several succeeding years in the study of medicine, with the plan in mind of returning to America to practice. This plan was given up, however, when in 1796 his resources were exhausted. After years of strenuous exploration following his son's return to France, André too sought the homeland. He sailed from Charleston in August, with the botanical materials and records that have made his name imperishable in the annals of American botanical science, but he was financially exhausted.

To add to his troubles, his boat was wrecked off the coast of Holland. He was rescued after floating on a board to which he had been tied, and in time much of his water-soaked botanical material also was rescued and dried out. Unfortunately, packages of priceless notes and records were lost, leaving gaps in the story of his strenuous life in the New World. He arrived in Paris after a time, where he was most favorably received, but the government was not interested in his plans for further explorations.

With the help of François, the year 1796 and several following years were devoted to the cultivation of the material from America, and in the preparation for publication of his luxuriously illustrated volume on the North American Oaks

and his volumes of the "*Flora Boreali-Americana.*" These publications set up a landmark in American botanical history.

André, still strenuous for the bright reward of additional scientific knowledge, through ministers and through the Central Society of Agriculture, tried to persuade Napoleon, then First Consul of the French Republic, to authorize further explorations in remote lands. Only after great effort was André appointed naturalist in a scientific exploration to Australian seas, under the command of Captain Baudin, it being agreed that Michaux should be permitted to remain at the Isle de France if he desired. After the freedom the uncontrolled explorer had enjoyed in his American years, he was disgusted with the haughty and discourteous manners of Captain Baudin toward the scientists associated with the expedition, and abandoned his commission at Mauritius. After six months, he decided that Madagascar offered a better chance to accomplish something for France and for Science, and set about establishing a nursery at Tametave for trees and other plants. He died there of fever in December, 1803.

François, now arrived at vigorous young manhood, sharing his father's objectives and feeling strongly as André had done that there was still much unfinished business in America, and relying on his experiences and acquaintanceships there, solicited a commission to resume work in the United States. De Chaptal, the Minister of the Interior, dissatisfied with the course of things in the nurseries still lingering near New York and near Charleston, gave François charge of them, with orders to ship to France such trees as still remained in them and to sell the land.

He set out on October 10, 1801, from Bordeaux for Charleston in the same boat, in charge of the same captain that had brought him home to France years before.

Thus, a rather remarkable thing was happening, the father who had rendered his name and that of France illustrious in the history of botany, was denied the opportunity to return to continue this work, being rather sent to his death in Madagascan wilds, while his son, with whom he had begun

scientific work in America, was sent back to carry on his father's work. As one sailed eastward in the naval vessel, the other took familiar passage to the west.

François arrived at Charleston on October 9, 1801, and spent much of the winter shipping stock from the Charleston nursery. He also visited Paul Saulnier, left in charge of the Hackensack nursery near New York. Saulnier, as a matter of fact, spent the rest of his life at this place, content to believe that he was still serving his master "Le Roi." He is said to have introduced the Lombardy poplar into America. If so, it remains a living reminder of that rare thing, a soul content to remain faithful over a few things.

François had accompanied his father on many long, wearisome and exciting journeys. Now he was to plan his own course and to travel much. He began botanizing in New Jersey and along the Hudson River. He discovered new oaks and hickories and sent the seeds to France. He did something that his father had done little of, in that he gave minute attention to the useful properties of the trees that his father was content to describe accurately and to name scientifically. He studied especially the oaks with tall trunks, good wood, and, in some cases, barks useful for tanning or dyeing. The lofty trunks characteristic of the American trees were greatly lacking in France. Of the forest trees of France, only 36 kinds reached a height of 30 feet. Of this number, 18 made up the bulk of the forests, and only 7 kinds were highly useful for civil and naval architecture. Among the American forest trees, 140 species reached the height required and many sorts offered the desired kinds of utility.

In June, 1802, he visited Philadelphia, meeting among others Collin, B. S. Barton, Vaughan, Peale and William Bartram. He is said to have enjoyed Bartram's Garden and William Hamilton's "Woodlands."

Having a few months at his disposal, he decided to go to Kentucky and Tennessee, regions that had won such high praise from his father. He set out on June 27, 1802, on his long journey. Going by way of Lancaster, Columbia, York,

Carlisle and Shippensburg, he crossed the Alleghenies, arriving in Pittsburgh in ten days, having traveled by stage, horseback and on foot. On July 14th, he set out for Wheeling, where he bought a canoe in which he descended the Ohio River in company with an American officer named Craff. On July 17th he was at Marietta, and on July 24th he landed at Limestone (probably now Maysville) in Mason County, Kentucky. From here he walked alone to Lexington, arriving there on August 10th. He continued on to Nashville, where he spent four weeks collecting in the vicinity or along the banks of the Cumberland River.

His return trip was to take him to Charleston. Starting on September 5, 1802, he entered upon a more interesting route than that followed in going westward. Fort Blunt, West Point and Knoxville, Falls of Roaring River and Jonesboro, in Tennessee, brought him to the mountains on the North Carolina boundary line. Soon he reached the farm of old Davenport, who had guided his father in those mountains in former years. He continued on by way of Morgantown, Winnsboro and Columbus, finally reaching Charleston, South Carolina, after a journey of 1800 miles, made in three and a half months. This trip furnished material for a book *Voyage á l'Ouest des Montes Allegheny*, published in 1804, and translated into English soon after. In this work one has an account not only of the vegetation, but comments on the conditions and methods of agriculture, on the nature of the soils, particular plant products, and the commercial relations between these backwoods regions and the seaboard cities.

On March 1, 1803, he sailed for France, landing at Bordeaux on March 26th. On going to Paris he found the publication of his father's volume on the oaks delayed. Although the text had been printed in 1801, the plates were still lacking. He also attended to the publication of his father's great "*Flora Boreali-Americana.*" He associated the botanist, Claude Richard, with the work in a literary capacity. Michaux's efforts at this period also resulted in the publication in 1804 of his own "*Voyage.*"

While in France, he took occasion to urge the planting of trees especially suitable to areas of that country lacking good native sorts. A communication to the Central Agricultural Society of France called attention to the magnificent timber trees found in the swampy lands of parts of America, and contrasted the willow oak, white cedar and cypress trees with the short, scrubby growth found in the wet lands of France. Sandy areas of America with the live oak so highly valued in naval construction were contrasted with unproductive sands of France. His communication in 1805 on the desirability of naturalization of forest trees of North America was warmly supported in a report to the Central Society of Agriculture by a committee consisting of De Pertuis, Cels and Correa de Serra, a member of this Society, at one time Portugese minister to the United States and temporarily at the head of the Department of Botany at the University following the death of Benjamin S. Barton. The plan proposed that a naturalist be sent to the United States to collect seeds and young trees of desirable sorts, to be sent to the national nurseries of France. The minister of finance, Duke de Gaete, favored the project, and François Michaux was entrusted with the responsibility of trying again to bring American trees to France.

He sailed from Bordeaux to Charleston on February 5, 1806, but after three days at sea, his boat was picked up by the British man-of-war "Leander" and Michaux was taken on board. After forty-three days afloat, he was landed on the Bermuda Islands, where he was allowed to go ashore. This detour in his route gave him a chance to study new plants and new scenes. These he described in a report "*Notice sur les Isles Bermudes.*" He was finally released and permitted to resume his journey. He was again in the United States near the end of May.

Now began a series of explorations covering much of the Eastern United States. Starting in the District of Maine, he traversed the states of the Atlantic coast as far south as Georgia, covering a distance of 1800 miles in this survey from

northeast to southwest. Along the coast he visited shipyards to see the timbers and methods used in American ship construction. He talked less with botanists and other scientists and more with artisans, and sought to meet the most skillful workmen in wood. He made five explorations into the interior.

He penetrated Maine along the Kennebec and the Sandy Rivers, perhaps to the Rangeley Lakes. He crossed New Hampshire and Vermont, going from Boston to Lake Champlain. Farther south he traversed the present state of New York from the city of that name to Lakes Erie and Ontario. Proceeding from Philadelphia, he reached the Monongahela, Allegheny and Ohio Rivers, and again following a route that must have been better known to him from his earlier travel, he went from Charleston to the sources of the Savannah and Oconee Rivers. In these journeys into the interior, he studied the trees making up the bulk of the forests, with special reference to the nature and uses of their woods, their commercial values in the exchanges between the states and in their relation to demands for exportation. Barks used in tanning, qualities and prices of woods, values for fuel, cabinet work and otherwise, were objects of his inquiry. A complete collection of polished specimens of different woods was made.

He remained in America about three years on this quest, making many new acquaintances among the prominent men of the day, Muhlenberg, William Hamilton, Barton, Hosack and Alexander Wilson being among the number. This may have aided in bringing about American recognition.

On April 14, 1809, Michaux was made an honorary member of the Philadelphia Society for Promoting Agriculture, a Society organized in 1785 to advance agricultural interests. The membership of this Society overlapped that of the American Philosophical Society in many instances, and for years the two organizations met in the same rooms. In the minutes of the meeting of the Philadelphia Society for Promoting Agriculture, held on January 10, 1809, Dr. James Mease, of Philadelphia, a member of this Society, proposed the name of

M. André Michaux, of Paris, as an honorary member, his election following at the meeting held on February 14th of the same year. Although he later lived near Paris, his interest in the affairs of the Society continued. Repeated references to communications received from him, pamphlets, books, and other publications, now on the shelves of the Society's library, offer substantial evidence of this interest.

On April 21st, of the same year, Michaux was elected to membership in the American Philosophical Society, an honor highly esteemed during the rest of his life. The Michaux Fund gives eloquent testimony on this point.

He now returned to France, presented himself to the Central Society for Agriculture that had sponsored his enterprise, and reported what he had done. The results of his efforts in America had been reaching France in shipments from time to time in the form of seeds and young trees. In this effort he had better success than his father. From the seeds sent over by the son, about 250,000 trees were obtained.

A committee was appointed with Corra de Serra as chairman to report to the Central Society on his effort. The report was most flattering. Michaux was complimented on the faithful execution of his trust, and a vote of thanks was passed in recognition of the important service rendered. With his great task of travel and physical hardship in a new and untamed world successfully accomplished and appreciated by his sponsors, Michaux, now about forty years old, still had long years before him in which to aid through efforts in France to bring to the homeland the solid result toward which the efforts of both his father and himself had been directed.

For two years following his return to France, he was busy in securing the publication of his great work "*Histoire des Arbres Forestiers de l'Amérique du Nord.*" Volume I appeared in 1810, volume II in 1812, and the third volume in 1813. The text was illustrated with 144 copper plates, designed by the eminent French artists, the Redoutés. In 1817, an English translation in four volumes by Augustus L.

Hillhouse was published in Paris under the title of "*North American Sylva.*" William Maclure bought the plates in Paris and brought them to the United States. American editions followed, the first brought out in New Harmony, Indiana, in 1842; a second appeared in 1852 in Philadelphia with notes by J. J. Smith.

After the appearance of his great work, Michaux devoted his attention to the cultivation and propagation of trees for public use in France. The Central Society of Agriculture still supported him by entrusting the use of a large estate to his care for experimental purposes. On his own country place near Pontoise and on the extensive lands of M. Delamarre, he carried on work of this character until his death. After a strenuous young manhood, the quieter life of the experimentalist must have had its rewards. He had the time and opportunity to carry to a successful conclusion the projects that had sent him to the wilds. He continued to take a special interest in the use of the so-called "heaths," of which France had at that time two million unproductive acres. As a result of forty years of experimentation by Michaux and the Central Agricultural Society, it was shown that these lands could be improved and used productively by the growing of "certain resinous trees," undoubtedly pines. This seems to be indicated by him in a letter written in 1852 to Isaac Lea, then President of the American Philosophical Society, in which he mentioned the Russian pine (*Pinus sylvestris*), known to us as the Scotch pine. He recommended this tree for special consideration in the northern and middle states of this country. It may seem somewhat strange that one who had studied the trees of this country and the conditions prevailing here, as Michaux had done, should have found any considerable use for this inferior species in the regions named.

In his letter to Isaac Lea, just referred to, written at the ripe old age of 82 years, he glances over the past. Speaking of his *Sylva*, he says: "The science of botany was the principal object of my father's explorations in North America and the

'Flora Boreali-Americana' was the result of these explorations. As for me, I took another view of the vegetable kingdom whilst in our country—a view more limited and less scientific, it is true, but perhaps more generally profitable to the farmer and the landholder, as well as to that class of society, so numerous in the Northern States of the Union, who employ wood in so many different ways. I do not consider my *Sylva Americana* as complete as it might be; thus, for instance, I have omitted several species which grow in lower Louisiana and in the two Floridas. In the second place, I have described and figured some trees that are deficient in the flowers and in the fruits. Had circumstances permitted, I would have returned to the United States, and, in a new edition, have corrected the errors and filled up the omissions. I would thus have been able to present to the American nation a work worthy of her great name, but now that I have arrived at a very advanced age—nearly 83 years —I can do nothing in this respect other than to express my regrets and the hope that some native arboriculturist may complete my researches on the plan which I have adopted. The publication of such a work would be attended with much benefit to the country, and afford particular honor to him who would undertake it."

Although he would be hardly called an arboriculturist, an English-born botanist, who also attained scientific fame in America, may have come in answer to Michaux's prayer. Thomas Nuttall, a botanical disciple of Benjamin S. Barton, of the Botanical Department of the University of Pennsylvania, later himself professor of botany at Harvard University, brought out a book in three volumes dealing with about 120 kinds of trees, mainly from the far west, a region not visited by Michaux. The two works supplemented each other in the way that Michaux had hoped, and the set of volumes makes a very important contribution to our knowledge of the forests of the United States a hundred and twenty-five years ago.

Michaux's interest in America dealt principally with trees,

but he concerned himself with other matters also. He happened to be planning a trip along the Hudson River in 1807, and hearing that a steamboat was to make its trial trip soon, found his interest aroused. He was pleased that he and another Frenchman were passengers when Fulton made the first trial trip of the "Clermont." That they were the only passengers may also have added to his satisfaction.

Not long before his death (1852), he added his contribution to the bulky literature that grew out of the terrible yellow fever epidemic that visited Philadelphia (in 1821) under the title of "*Causes of Yellow Fever in the United States.*"

Michaux's life as a bachelor ended in a rather sudden decision at an advanced age to marry his housekeeper, a remote relative who had long managed his household. Elias Durand, a Frenchman residing for a time in America, who had known Michaux in his later years, and who had aided him by collecting and forwarding American tree seeds to France, writes in his account of Michaux, published in the *Transactions of the American Philosophical Society* in 1890, that he was carried off with frightful suddenness by a stroke of apoplexy on the 23rd of October, 1855, "after a busy day among his American trees."

He was in comfortable financial circumstances, and deposited a will with Isaac Lea four years before his demise, providing for the now well-known Michaux Fund, to be used by the American Philosophical Society after his wife's future had been provided for.

After the death of this lady, the fund was used in part for the creation of a monument to the generous donor, and this monument still exists as the Michaux Memorial Grove of oaks in Fairmount Park. This grove was planted near Horticultural Hall at the opening of the Centennial Exhibition in 1876, when Fairmount Park was in the earliest stage of its formation. The Chairman of the Committee on Trees and Nurseries in the Fairmount Park Commission, Mr. Eli Kirk Price, who was also chairman of the Committee on the Michaux Fund in this Society, reported to the Society on

June 16, 1876, that "he had visited today the Fairmount Park nursery and found the ground well taken care of, and the large stock of trees in a flourishing condition. Of the acorns planted last winter, nearly all have grown except those of the 'Bartram Oak,' and of the fifty or sixty of those planted not one has grown. So far the proof is in favor of that oak being a hybrid."

It seemed to me a matter of interest at this time to see what has happened to this Grove in the sixty or more years since it was established.

Accordingly, the area indicated to be that occupied by the Grove on a map of the Park prepared for the Centennial Exhibition of 1876, shown us by Mr. S. N. Baxter, the present Arboriculturist of the Commission, was visited and the ages of a number of trees now present determined by the use of an increment borer. Mr. R. D. Forbes and his assistant, Mr. O. M. Wood, both of the Allegheny Forest Experiment Station, made the test on trees selected by Mr. Baxter as representative specimens. Out of 7 tested, 6 showed an age estimated to be 60 years. These trees undoubtedly belong to the Michaux Grove. From this examination it seems clear that the Memorial Grove is still in large part made up of the oaks reported on by Mr. Price in 1876 and, judging from the uniformity of the Grove, relatively few of the original trees have had to be replaced.

Michaux Avenue now passes through the Grove and presents one of the most beautiful spots in the Park. Thus, the living memorials of these friends of trees are still in their prime, and, if spared by weather, disease and other hostile influences, should delight generations still to come before old age in the end brings them down.

In closing this sketch, I will take the liberty of making some suggestions regarding ways in which the Society may continue to carry out the will of the donor, and at the same time advance the significance of the organization.

Michaux was an ardent advocate of a public policy of support for the scientific study of wild plant life. He studied

it with equal interest with reference to human utility. Probably influenced to a considerable extent by the needs of France at that time, his special efforts were centered on trees in nature and under cultivation. Something perhaps remains in the forests of France of the trees introduced by the Michauxs. How much they actually did for their country in this respect I do not know. For us and for the rest of the world, their greatest accomplishment is seen in the writings they have left. With the world changing as rapidly as it is, authentic records of the past should be sought out and held with great care by repositories like this Society. Hence, accounts of explorations and records of earlier days in the fields and forests are basic material. As the scene changes, succeeding accounts will continue the story.

For example, the question of soil conservation, a very old one and now much in the public mind, is presenting itself as a series of problems in agriculture, forestry, wild life preservation and human welfare in all of their many aspects. Old records tell of nature and of early human life. We should have those early records. The story should be continued by the important writings of later days. Thus, the early contributions of the Michauxs, father and son, remaining in our library, become a priceless possession as the record continues to unfold.

Memorial trees in time die and their places are forgotten. The practical accomplishment of the past must be sought in books and other lasting sources of information. Exploration, forestry, botany, plant introduction and plant utilization, the problem of the soil that supports all life, are subjects that may fittingly claim the earnest attention of the Society in building up and preserving its collection of records. Utility may be immediate. Certainly there will be a priceless value for the future if civilization persists and present ideas of worth continue to be held.

Reprinted from THE BRYOLOGIST, Vol. XL, July-August, 1937

LICHENS FROM THE NEW ENGLAND COAST

RODNEY H. TRUE

Some years ago, the writer spent parts of two summers on the islands of Casco Bay and collected a few specimens of lichens that were sent to Dr. C. C. Plitt, of Baltimore, for identification. Fortunately, the list of names given to them was received in Dr. Plitt's lifetime. The specimens named have been

deposited in the herbarium of the Department of Botany of the University of Pennsylvania.

The list that follows is prepared from Dr. Plitt's manuscript. The several locations at which collections were made with dates are indicated, also the numbers of the several collections given by the collector.

Orr's Island, Cumberland Co., Maine

Acarospora fuscata (Schrad.) Th. Fr.—On mica schist rocks. (*815*) Aug. 19, 1922.
Baeomyces roseus Pers.—On the ground in woods, toward Cow Cove. (*773*) July 21, 1922; near head of Long Cover (*813*), Aug. 15, 1922.
Cladonia crispata (Ach.) Flt. f. *infundibulifera* (Schaer.) Wainio.—On decaying stump, dry ridge. (*955*) July 30, 1923.
Cladonia cristatella (Tuck.)—On decaying wood and humus soil in dry clearing. (*946*) July 30, 1923.
Cladonia fimbriata (L. f.) var. *cornuto-radiata* Coem. (probably).—On rich earth near low shrubbery. (*950*) July 30, 1923.
Cladonia gracilis (L.) Willd. var. *elongata* (Jacq.) Flk.—Among mosses on mica-schist rocks near Great Island Bridge. (*860*) Aug. 21, 1923; (*984*) on dry shaded rock Aug. 20, 1923.
Cladonia pyxidata (L.) Fr. var. *neglecta* (Flk.) Mass.—Rich soil, in edge of woods (*951*), July 30, 1923.
Cladonia rangiferina (L.) Webb.—On dry ground under spruces (*867*). Aug. 24, 1922.
Cladonia reticulata (Russell) Wainio.—On dry rocks, ridge east of village (*835*), Aug. 19, 1922.
Cladonia squamosa (Scop.) Hoffm. var. *denticollis* (Hoffm.) Floerke.—On rich earth under shrubbery (*947*), (*958*) July 30, 1923.
Cladonia squamosa (Scop.) Hoffm. var. *muricella* (Del.) Wainio.—On dry soil and rocks under spruces, path to David Orr's Cove. (*866*) Aug. 24, 1922.
Cladonia sylvatica (L.) Rabenhorst f. *laxiuscula* Del.—On dry rocks (*814*) Aug. 19, 1922.
Cladonia turgida (Ehrh.) Hoffm.—On dry rocks under spruces, ridge on path to David Orr's Cove (*865*), Aug. 24, 1922.
Cladonia uncialis (L.) Web.—On dry soil, top of ridge (*944*), (*945*), (*949*) July 30, 1923; (*868*), (*877*), path to David Orr's Cove Sept. 7, 1922.
Cladonia verticillata Hoffm.—On decaying wood and leaf mold (*952*), July 30, 1923.
Diploschistes scruposus (L.) Norm.—On dry mica-schist rocks (*834*), Aug. 19, 1922.
Gyrophora Dillenii (Tuck.) Müll. Arg.—On mica-schist rocks near Great Island Bridge (*858*), (*862*), Aug. 21, 1922.
Lecidea convexa (Fr.) Th. Fr.—On mica-schist rock, east of village (*818*), (*820*), (*821*), (*826*), (*830*), (*831*), Aug. 19, 1922.
Lecidea granulosa (Hoffm.) Ach.—On dry mica-schist (*823*), Aug. 19, 1922.
Leptogonium tremelloides (L. f.) Gray.—On mica-schist ridge, east of village. (*819*), Aug. 19, 1922.
Lobaria pulmonaria (L.) Hoffm.—On trunk of maple tree (*872*), Sept. 4, 1922.
Parmelia conspersa (Ehrh.) Ach. f. *isidiata* Leicht.—On mica-schist rocks, ridge east of village (*825*), (*833*), Aug. 19, 1922.
Parmelia physodes (L.) Ach.—Common on branches of spruce (*960*), July 30, 1923; (*969*), Aug. 29, 1922.
Parmelia saxatilis (L.) Fr.—On bark of spruce tree (*871*), Aug. 29, 1922.
Parmelia sulcata Tayl.—On mica-schist rocks (*836*), Aug. 19, 1922.

Peltigera aphthosa (L.) Hoffm.—On damp shaded soil among mosses, path to David Orr's cove. (*885*), Sept. 7, 1922.
Peltigera polydactyla (Tuck.) Hoffm.— Dry soil under blueberries. (*956*), July 30, 1923.
Physcia obscura (Ehrh.) Th. Fr. var. *endococcina* (Kbr.) Th. Fr.—On mica-schist rocks. (*819a*), August 19, 1922.
Pertusaria amara (Ach.) Nyl.—On mica-schist rocks. (*816*), Aug. 19, 1922.
Rhisocarpon petraeum (Nyl.) Zahlb.—On dry open mica-schist ridge. (*817*) (*824*), (*827*), (*828*), Aug. 19, 1922.
Stereocaulon paschale (L.) Fr.—On mica-schist rocks. (*829*), Aug. 19, 1922.
Umbilicaria pustulata (L.) Hoffm.—On mica-schist rocks near Great Island bridge. (*861*), Aug. 21, 1922.

Scattering collections were made at other points in Casco Bay during the same years. Determinations were also made by Dr. C. C. Plitt:

Cladonia gracilis (L.) Willd. var. *elongata* (Jacq.) Flk.—Dry hillside, north of entrance to New Meadows River Basin, Sagadahoc Co., Me., July 18, 1923, (*936*).
Cladonia pyxidata (L.) Fr. var. *chlorophaea* Flk.—Moist shade. Indiantown Island, Sagadahoc Co. (*1006*), Sept. 1, 1923.
Cladonia rangiferina (L.) Web.—Dry hillside, north of entrance to New Meadows River Basin, Sagadahoc Co., Me. (*926*), July 18, 1923.
Cladonia reticulata (Russell) Wainio.—On the ground, Small Point Harbor, Cumberland Co. (*993*), Aug. 23, 1923.
Cladonia squarrosa (Scop.) Hoffm. var. *denticollis* (Hoffm.) Flk.—Under spruce trees, Ragged Island, Cumberland Co. (*768*), July 15, 1922.
Cladonia uncialis (L.) Web.—Open dry ground, Small Point, Sagadahoc Co. (*997*), Aug. 23, 1923.
Gyrophora Dillenii (Tuck.) Müll. Arg.—On mica-schist outcrop, Small Point Harbor, Sagadahoc Co. (*1002*), Aug. 23, 1923.
Umbilicaria pustulata (L.) Hoffm.—On shaded rock face, entrance to New Meadows River Basin, Sagadahoc Co. (*923*), July 18, 1923.
Usnea dasypoga (Ach.) Nyl.—On dead spruce, entrance to New Meadows River Basin, Sagadahoc Co. (*937*), July 18, 1923. On branches of conifers, Ragged Island, Cumberland Co. (*772*), July 17, 1922.

Nantucket Island

During a brief visit to Nantucket Island in 1923, a small collection of lichens was made. The names by Dr. Plitt and numbers follow:

Cetraria islandica (L.) Ach.—Dry soil. (*967e*), Aug. 8, 1923.
Cladonia alpestris (L.) Rabenhorst.—Common on dry sandy uplands. (*961*), Aug. 5, 1923.
Cladonia cristatella Tuckerman.—On dry sandy soil of Commons. (*962*), Aug. 5, 1923.
Cladonia furcata (Huds.) Schard.—Dry soil of Commons. (*964*), Aug. 5, 1923.
Cladonia rangiferina (L.) Web.—Common on soil of dry uplands. (*963*), Aug. 5, 1923; (*967c*), Aug. 8, 1923; (*967d*), Aug. 8, 1923.
Cladonia reticulata (Russell) Wainio.—Dry soil of Commons. (*967*), Aug. 5, 1923; (*967f*), (*967g*), Aug. 8, 1923.
Cladonia verticillata var. *evoluta* Th. Fr.—Dry soil of Commons. (*967a*), Aug. 8, 1923.

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IRREGULAR PAGINATION

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GALL DEVELOPMENT ON PINUS SYLVESTRIS ATTACKED BY THE WOODGATE PERIDERMIUM, AND MORPHOLOGY OF THE PARASITE[1]

R. P. TRUE[2]

(Accepted for publication Aug. 26, 1937)

INTRODUCTION

A *Peridermium* attacks Scotch pine, *Pinus sylvestris* L., plantations bordering Round Lake near Woodgate, New York, causing susceptible trees to form globoid galls. Since its discovery there by York (13) in 1925, it has been called the Woodgate rust or the Woodgate *Peridermium*. York's observations led him to believe that the rust has been present since 1895 in these plantations, some of which, planted in 1870, are among the oldest in America. Some trees of *P. sylvestris* do not form galls after natural infection or inoculation with the fungus. Hutchinson (6) has recently investigated this problem of resistance. The present study deals with the reactions of susceptible trees and the morphology of the rust fungus.

INOCULATIONS AND FIELD NOTES

One hundred forty field inoculations were made upon 27 trees of *Pinus sylvestris,* ranging in age from 9 to 30 years and located in the infection area, supplemented the naturally infected material available for study. The inoculated trees varied widely in susceptibility, as indicated by the varying number of galls they bore from natural infection. The inoculations were made between the 7th and 30th of June from 1930 through 1933. Susceptibility was found to be highest during the first half of June and to decline toward the end of the month.

The aecial inoculum was obtained from fruiting galls collected in the vicinity. Spores for immediate use were carefully removed from the galls as soon as the latter were collected. Galls whose spores were to be used in later inoculations were placed indoors in a dry shaded place and the viability of the spores was tested periodically by placing samples of the spores on fresh well water and noting their percentage germination. Inside storage was resorted to because the spores of galls left late in the field often are blown away or washed out, or they germinate in the aecium.

The method of inoculation was that employed by York,[3] using the celluloid iceless refrigerator. Early inoculations were made upon the stems of the current season's shoots at the time when their elongation separated the developing needles sufficiently to permit a direct inoculation upon the surface of the stem. The needles borne on the young shoots had elongated to more than half their mature size before the last inoculations were made.

Field observations and collections of artificially and naturally infected materials were made each year. During the summer of 1933, observations were recorded and collections made daily for periods of from 3 to 7 days after inoculation and thereafter at weekly intervals. In other years, after the month of June, the intervals between collections varied from 1 to 2 months until dormancy. Notes and collections also were made in late November and in May to reveal the developmental stages present shortly after entry of the host into the winter condition and its breaking dormancy.

ANATOMICAL STUDIES

Methods

Newly inoculated shoots, developmental stages, and mature galls were fixed in the field. Bouin's solution, formal-acetic-alcohol, and formalin-alcohol proved the most satisfactory of the 13 fixatives tried. The first and the last gave the best fixations of the fungus. During fixation, air was removed from the tissues by placing the vials containing the specimens in a closed chamber and evacuating it with a suction pump.

After 12 to 48, and, in some cases, 72 hours of fixation, most of the material was dehydrated and transferred into pure hard paraffin by Zirkle's (14) butyl-alcohol method. Tissues not imbedded at once were stored in the dehydrating mixture made up of 30 parts of water and 50 to 20 parts of ethyl and butyl alcohol, respectively, where they remained reasonably soft until dehydration could be completed.

Early infection and developmental stages for histological study as well as three-year old galls were cut from 8 to 15 μ thick. Material with sori in the bark was cut as thin as 3 μ. Land's method, using a saturated solution of gum arabic followed by a 1 per cent potassium dichromate solution to float out the ribbons, was found most satisfactory in fixing the sections to the slide.

The stains that most clearly differentiated the mycelium in its relation to the host tissue were Orseillin BB in a 3 per cent aqueous solution of acetic acid, counterstained with a similarly acidulated aqueous solution of anilin blue, or with a saturated solution of crystal violet dissolved in clove oil. By following a procedure recommended by Doak,[4] crystal violet in clove oil was used alone to show excellent detail in hyphae and haustoria, but it often necessitated destaining the host tissues too far to show them clearly. The combination of Heidenhain's haematoxylin and iron alum was used to show nuclear phenomena and, in combination with safranin, to show the histological details of the gall.

[1] A dissertation submitted to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] The writer is indebted to Dr. H. H. York for his advice and criticism during the progress of this work, and to Dr. Conway Zirkle and Dr. K. D. Doak for many helpful suggestions. He also wishes to acknowledge the aid of the Division of Forest Pathology of the Bureau of Plant Industry of the United States Department of Agriculture. The photographs were made with the help of Mr. Vernon Doney with the exception of figure 1, C, which is from one of Dr. H. H. York's negatives.

[3] York, H. H. Celluloid "iceless refrigerator" and some results from field inoculations with the aeciospores of Woodgate rust (*Peridermium* sp.). [Manuscript.]

[4] Personal communication.

IRREGULAR PAGINATION

For field study, the following rapid method was used to obtain permanent slides: Fresh material was cut in water on a portable sliding microtome and placed at once in Bouin's fixative, where it remained for 10 minutes. It was then washed in water until no further color of the fixative came from it and set to stain for five minutes in Orseillin BB. Excess stain was washed out in the early steps of dehydration and, after a brief clearing, in clove oil followed by xylol, the material was mounted in balsam. The whole schedule may be completed in from 30 to 45 minutes.

External Morphology of Gall Development

In order to make it easier to follow the sequence of internal events described under histological studies, it seems desirable to give a brief summary of the external phenomena that often follow infection and accompany gall development, as observed in inoculated material and supplemented by observations of galls resulting from natural infection. While the phenomena here considered often occur as described, gall formation is very variable and the times and sequence of events show such variation that this brief outline must be considered only as an aid to the understanding of the histological phenomena involved.

Infection occurs primarily through the epidermis of the current season's shoots. Points of infection often are marked by spots that usually range from orange to dark brown. These sometimes appear in the first week of July. Their margins are often sharply defined, but frequently are less definite, and may appear water-soaked. Infection spots do not necessarily indicate successful invasions. Infections whose spots show indefinite or water-soaked margins seem more apt to produce galls than those with more sharply delimited spots. As observed, slight swellings may result in the season of infection below many infection spots and spot-free points of infection. In the second season, dead shrunken cortical areas may appear in the central portion of the surfaces of the swellings, which by the end of that season may become distinct spherical or hemispherical galls. In May of the third season, two years after infection, slight colorless exudates are at times found near the margins of the sunken areas, which, in some cases, may be associated with internal pycnia. Toward the end of the third season, the central sunken area occupies most of the gall surface, and is bounded by a slightly raised rim.

During the fourth season the gall usually acquires those external characteristics which typify it during its further enlargement (Fig. 1, A). Aecia are produced in the central sunken areas of the roughly spherical or hemispherical galls (Fig. 1, D). As the aecia develop, they push up the dead tissues, which usually scale off, eventually exposing the often confluent fruiting surface (Fig. 1, C). Since the layer of aecia-bearing tissue is in turn usually exfoliated before winter (Fig. 1, E), the central portion becomes more and more sunken in comparison with the peripheral rim, which then appears as a collar delimiting the gall (Fig. 1, A, C, and E). On an occasional tree, the periderms of the galls are not sloughed off but, as swelling continues, are cleft by deep cracks in which the aecia may be borne. (Fig. 1, F.)

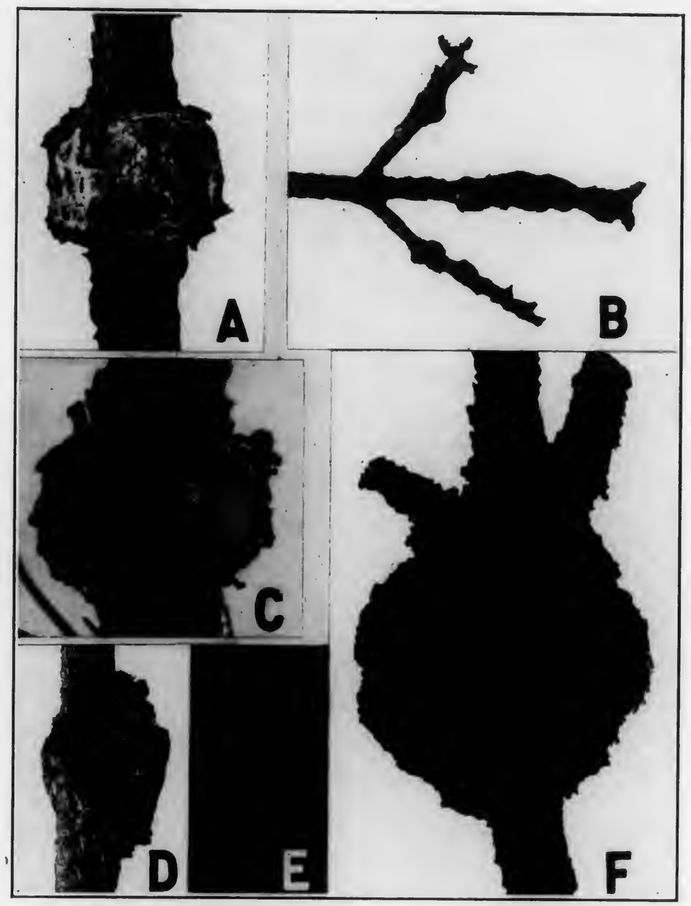

FIG. 1. A. Typical spherical gall with sporulation well advanced. B. Young fusiform swellings due to multiple infections. C. Gall with sporulation completed. Note complete exfoliation of periderm external to aecial layer. D. Hemispherical gall. E. Gall with aecial layer being sloughed away by a new periderm formed benath. F. Ribbed gall with adhering periderm.

Galls on an occasional tree become necrotic and frequently develop abortively, or are misshapen, often through necrosis originating in their central areas, which then appear much more deep-sunken than in normal galls. Such necroses may well at times be caused by invading secondary fungi. Mass infections resulting from inoculations of twigs of highly susceptible trees may cause an extensive killing of the outer layers, which, with subsequent swelling, crack deeply into the woody tissues forming longitudinally

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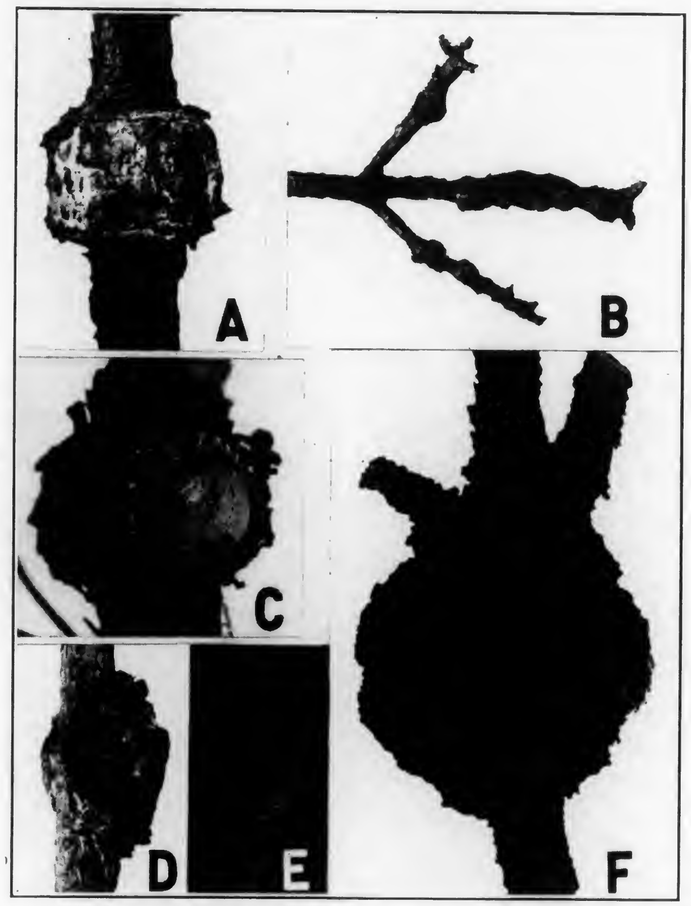

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INTENTIONAL SECOND EXPOSURE

confluent furrowed swellings. These sometimes fail to produce galls or develop into roughly fusiform compound galls (Fig. 1, B), which fruit very sparsely, if at all.

Histology of Invasion and Host Reaction

Epidermis and Primary Cortex. Most successful infection occurs preceding periderm formation. During this period the stem is protected by an epidermis whose outer walls are, in general, heavily lignified and are covered with a thick cuticle (Pl. I, 1). At the bases of needle fascicles, however, walls of the epidermal cells are uniformly thick, apparently uniformly lignified throughout, and usually are pitted. At the fascicle bases, the outer walls of even the subepidermal cell layer become lignified and sunken stomata appear. The cells of the first subepidermal layer are large and radially elongate (Pl. I, 1). Other nonspecialized cells of the cortex appear to be typical of parenchyma tissues in general. Cells of the resin ducts found in the inner cortex are vertically elongate and often contain tannin-like substances, as do a few lignified cells with pitted walls, which are scattered through the cortical parenchyma. About the middle of June a meristem arises from the subepidermal cells or from the cell layers beneath, forming 2 or 3 rows of thin-wall, tangentially elongate cells, the phellogen, external to which the cells die and gradually collapse (Pl. III, 7).

All infections, observed histologically, have been made through the epidermis, either inter- or, more frequently, intracellularly, in the less lignified portions. Appressoria have not been noted. (Fig. 3, D). The germ tube penetrating the cuticle and outer walls of the epidermal cells is considerably narrowed, but, once inside, it broadens, forming a vesicle, and may branch before leaving the cell to enter the subepidermal intercellular space (Fig. 3, A). In the cases of cells penetrated by germ tubes, the thickening of the outer walls was at times much less pronounced than in unpenetrated neighboring epidermal cells. This seems not to be a matter of selection by the fungus, since no unpenetrated walls of comparable thinness were found in the same or similar material. If it is a result of fungus invasion, whether of actual dissolution of the wall or of preventing its normal thickening, it is produced quickly, since it is already obvious in material only 16 days after inoculation. Once the infecting hypha passes from the epidermal cell into the intercellular space below, it branches readily and sends hyphae into neighboring epidermal and subepidermal cells. These hyphae appear to function as haustoria, but, unlike the typical haustoria formed deeper in the cortex, they are often septate and may even branch (Fig. 3, B). This tendency toward intracellular development is short-lived, and the mycelium soon establishes itself intercellularly with typical haustoria.

While trees upon which galls develop are here considered susceptible, a large percentage of the infections fail to result in gall formation, even on the most susceptible trees. Histological studies of early stages of the invasion of such trees have shown that, while some infections meet with no apparent

FIG. 2. A. An invasion resisted partially by cell hypertrophy as at A. B. An invasion spreading vertically along the resin canal (A) has stimulated the adjacent tissues to cell division and radial cell orientation accompanied by marked reduction of intercellular space. C. An invasion being effectively walled out by a cicatrizing zone. D. Radial longitudinal section of infected phloem and xylem. Note broad rays with abundant intercellular hyphae (A); short, blunt irregularly pitted tracheids (B); abundance of phloem parenchyma (C); and resin-containing cavity (D) associated with radial resin canal not shown in this section. E. Transverse section showing wedge-shape area of gall wood with adnormalities intensified at the edge. Note also the internal and external areas of killing in the cortex. F. Longitudinal section showing a late stage in the development of the "collar" with living tissues considerably isolated radially by the death of cells interior to them.

resistance, others are constantly opposed by partial barriers, and yet others may at once be surrounded by barrier zones and walled out completely. In the course of the present study, a shoot no more than 2 cm. long, sectioned serially a month after inoculation, was found to have 24 infections. Five of these showed no trace of recognizable mycelium among the very abnormal

confluent furrowed swellings. These sometimes fail to produce galls or develop into roughly fusiform compound galls (Fig. 1, B), which fruit very sparsely, if at all.

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INTENTIONAL SECOND EXPOSURE

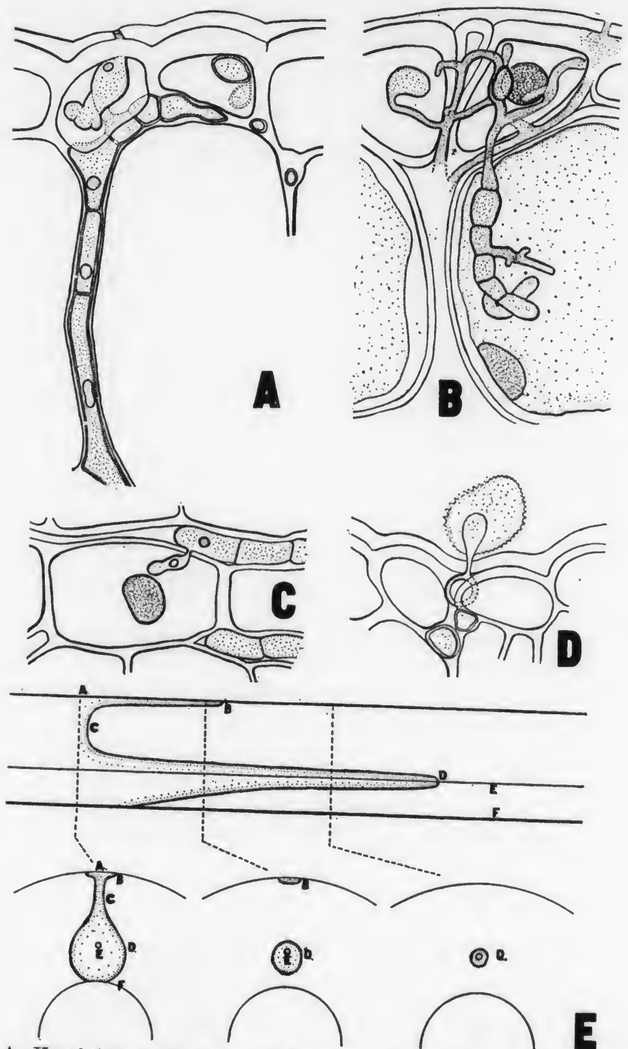

FIG. 3. A. Usual intracellular type of penetration showing vesicle in epidermal cell and subsequent intercellular development. B. Branching septate haustorium within a subepidermal cell. C. Mycelium and haustorium in the phloem parenchyma. D. Spore with short germ tube entering host, showing absence of appressorium and the intercellular type of penetration. E. Diagrammatic representation of the spread of the mycelium of a typical unresisted infection through the primary cortex. Radial longitudinal spread is shown and the appearance of transverse sections taken at various levels is indicated: A, point of penetration; B, extent of subepidermal spread; C, path of radial growth; D, extent of spread adjacent to resin duct in inner cortex; E, cortical resin duct; F, the phloem.

and often dying cortical cells. Most of them showed isolated groups of living hyphae penetrating the more normal portions of the cortex and the intercellular remains of others where tissue abnormalities were more severe. In the case of one infection, discovered in sectioning, where no external symptom was present, the abundant and continuous spread of the large intercellular mycelium had produced almost no abnormality in the areas it penetrated. This infection had penetrated as deeply into the cortex as most of the others, some of which were still confined to the outer cortex.

Infections to which little or no resistance apparently is offered are those that cause but slight early abnormality in the host. The abundant large hyphae pass between or encircle nearly every cell in the infected area (Pl. I, 2 and 3), and many cells are penetrated by the ellipsoid to cylindric haustoria, which are uninucleate and greatly attenuated where they pass through the cell wall (Fig. 3, C and Fig. 6, E). The haustoria often appear to seek out the nucleus of the host cell and those touching it appear at times to be flattened at the point of contact. The host cells in the infected zone are more spherical and regular in outline than those in the normal cortex, and there is a tendency toward hypertrophy, which is most pronounced in the cells invaded by haustoria.

The hyphae of a mycelium whose invasion apparently is unresisted advance in characteristically different directions (Fig. 3, E). Some run parallel to the axis of the twig in the spaces between the first one or two cortical cell layers or just beneath the epidermis. The major portion of the mycelium, however, spreads radially inward through the outer cortex until the inner cortex is reached, and there a second vertical spread occurs, which is most pronounced in the intercellular spaces adjacent to and near the epithelial cells of the resin ducts. These cells often show a tendency to hypertrophy, increasing in size until they may partially or completely fill the ducts. (Pl. I, 2). The mycelium often spreads a considerable distance about the margin of the phloem before entering it. (Pl. I, 3).

Fresh shoots of the current season's growth, collected September 20, 1933, and tested microchemically, indicated a possible physiological explanation for the diversified paths followed by the mycelium in unresisted infections. The layer of cells just below the phellogen, and at times the cells of the phellogen itself, well established at that date, were found containing starch, particularly near the bases of the needle fascicles, where every cell was filled with it. Elsewhere in these peripheral tissues starch was less abundant, appearing only in scattered cells. Internal to this layer the cells of the outer cortex seldom showed starch except below needle fascicles where starch-containing cells are few and scattered. The inner primary cortex, however, had numerous scattered starch-containing cells in the vicinity of the phloem and near the resin ducts. Droplets of fatty substances were present in scattered cortical cells, but were especially abundant in the cells surrounding the resin ducts. Compounds giving a positive reaction to the ferric chloride test for tannins were found in scattered cells in the inner cortex, but especially in the

cells bordering the resin ducts, where they appeared as coarsely to finely granular and, at times, even as amorphous material.

The presence of these substances, at least two of which may be associated with nutrition, in those tissues where the growth of the mycelium is most rapid, suggests that the localization of foodstuffs within the host may influence the paths of invasion in the cases of unresisted infections.

Partially resisted invasions tend to follow the same paths as those whose progress is unopposed, but they meet with localized mechanical resistance from the host, which often alters the direction and curtails the extent of their spread.

Three mechancial responses offer some degree of resistance to the invading mycelium. The first is exaggerated hypertrophy of the cells invaded and often a less pronounced enlargement of contiguous cells (Fig. 2, A). This diminishes the space between the cells, opposing the intercellular proliferation of the mycelium. Close-pressed hyphae in varying stages of abnormality have been observed wedged between the walls of enlarged cells in areas of severe cell hypertrophy. In other cases the advancing hyphal tips may grow beyond the enlarging cells before the extreme stages of hypertrophy are attained and so escape this means of mechanical resistance. The difference in degree of hypertrophy in the invaded host cells seems to be one measure of the compatibility of the reacting organisms.

A second structural response usually offers some resistance to invasion and, like excessive cell enlargement, seems to indicate considerable incompatibility between the host and the invading mycelium. Mature cortical cells in and near the infection area divide, filling the space they formerly occupied with a larger number of smaller cells often oriented in rows radiating from the infected area (Fig. 2, B). The radial arrangement of the cells might appear to favor a swift invasion, but it usually is accompanied by the almost complete elimination of intercellular space, which again appears to be the morphological barrier to further invasion. Both hypertrophic and hyperplastic reactions appear to be direct responses to the invading fungus.

The host often responds structurally to invasion in a third way. A row of phellogen cells derived from mature elements of the primary cortex produces a distinct zone of cicatrization next to infections in which host cells have been killed. This layer cuts off the dead cells, together with some of the very abnormal cells adjacent to them, from the more normal tissues. This cicatrix often serves as a successful barrier against further invasion but does so only when the host cells attacked die so swiftly that the cicatrizing zone walls out the invading mycelium before it has had time to spread (Fig. 2, C). In case of an infection by a mycelium fairly compatible with the host, invaded areas remain nearly normal for some time after invasion during which the invading mycelium spreads deep into the host tissue before any of the invaded cells die, stimulating cicatrization (Pl. II, 2).

Microchemical tests made upon material showing partially resisted invasions revealed that abnormalities in the visible cell contents appear in tissues before they show the structural deviations described above. Starch is unusually abundant in the early stages of physiological abnormality. It is present in large quantities in the morphologically normal cells at the margin of the infection, both before and after the tissue has been invaded and its cells penetrated by haustoria. The presence of these large deposits of starch within and adjacent to the infected areas is accompanied by a scarcity of it elsewhere in tissues in which it normally occurs. Fatty globular substances appear abnormally abundant in the pathic living cells and seem abnormally scarce in adjacent uninvaded tissues. Tannin or tannin-like substances are universally found in cells that have passed the early stages of abnormality; and their appearance often is synchronized with the complete disappearance of starch and fatty substances from the pathic cells. They appear in the wall or lumen of nearly every dead cell.

In the case of barrier formation, the thin-wall cells of the barrier are not cutinized nor lignified, nor do they contain starch, fatty substances, tannin, or tannin-like compounds. They give a negative reaction to tests with Scarlet R; but Hutchinson (6), using ammoniacal gentian violet as a reagent, found them to be suberized in the material with which he worked. A globular fatty metabolite, staining pink with Scarlet R, is often present in the meristematic cells below the barrier, and scattered cells of the adjacent cortex, and starch grains appear similarly distributed.

Whether the invasion is resisted or not, invaded cortical tissues eventually die. Their death occurs generally in the same chronological order as their invasion, so that, in cases of at least moderate compatibility, an external area of killing appears close beneath the epidermis to correspond with the early vertical spread of the mycelium there, and a similar but more extensive internal area is located in the inner cortex, where the internal vertical proliferation took place. These are united opposite the point of epidermal penetration by a smaller area of killing in the outer cortex, where the centripetal spread occurred. (Fig. 3, E).

External areas of killing usually involve the epidermal cells, as well as those directly interior to them, and are clearly visible to the naked eye as infection spots. When these spots are minute or have sharply defined margins, it may be taken as an indication that the invasion is being at least partially opposed by the host; particularly, if they appear within a month or six weeks after inoculation. Larger spots having indefinite margins often appearing water-soaked are likely to mark successful infections, and especially so if their appearance is considerably delayed. In cases of extreme compatibility, swellings may appear before their infection spots. Most infection spots on susceptible trees at Woodgate show intermediate characters.

Peripheral and longitudinal spread of the fungus is limited, at first, almost entirely to the primary cortex, and, later, to the phloem. The mycelium spreads more swiftly in the inner than in the outer cortex, and the death of the invaded cells of the former cuts off the partially invaded and

mostly living cells of the latter from radially conducted water and nutrients. (Pl. I, 4). Thus isolated, they in turn die and collapse against the dead inner cortex, forming a sunken area at the surface of the central portion of the swelling. The peripheral areas of the outer cortex, only partially, or not at all so isolated, remain alive for some time as a rim or collar. This becomes more pronounced after the middle or the end of the second season when the infected cambium usually has cut off considerable phloem tissue, which serves thereafter in the place of the cortex as the chief region of vertical and peripheral spread of the mycelium. In the third season, on fruiting, the dead tissues of the central sunken area are sloughed away, making the collar even more prominent (Fig. 2, F). In the enlarging gall this collar gradually dies back as its edges are further isolated by the continual dying of the tissues internal to it, but it remains distinctly prominent in the case of most mature galls, even after several years.

While responses of resistance often fail to prevent gall formation on susceptible trees, they may so limit peripheral spread of the organism that a hemispherical rather than a spherical gall is produced (Fig. 1, D), or they may hold the mycelium in the cortical region for one or more seasons, thus delaying gall formation. The mycelium of an unresisted infection was noted penetrating the cambium on July 20 of the season of infection and, by August 1, the hyphae of several of that season's infections, some of which had been resisted, had penetrated beyond the cambium. One case was noted in which a resisted infection was just entering the phloem on June 15 of the season following that of its epidermal penetration.

Matured Stelar Tissues. The mycelium passes through the intercellular spaces of stelar tissue derived from uninvaded cambium largely in a radial direction. The structural response of the cells of phloem tissue to the presence of the fungus is limited mostly to cell enlargement, but a division of some of the matured cells at right angles to the line of invasion, and even the formation of a barrier, have been observed in exceptional cases. Penetrating mostly along the phloem rays, the mycelium crosses the cambium at right angles and continues its radial path mostly along the medullary rays for but a short distance into the xylem, where it fails to cause any appreciable structural abnormalities.

Like the cortex, however, the stelar tissues may undergo a marked physiologic response because of the presence or even the proximity of the fungus. Starch grains and fatty droplets, present to some degree in the normal parenchymatous cells of both phloem and xylem, are often present there in abnormally large amounts in tissues adjacent to, as well as within, infected areas.

Cambium. While the fungus usually causes but slight structural abnormality in the matured phloem and apparently none in the xylem, its presence stimulates the cambium to the production of definite and characteristic abnormalities in the phloem and xylem formed after cambial invasion. In view of Bailey's (3) work, indicating that the length and volume of tracheids of normal gymnosperms are closely correlated with the length and volume

of their cambial initials, it seems likely that these abnormalities reflect abnormalities in the cambial initials induced by the presence of the fungus in the cambium.

The cambium usually is first penetrated at a point or over a limited area adjacent to the point of infection, and subsequent cambium invasion comes always from the phloem, never from vertical or peripheral spread of the mycelium within the cambium itself (Pl. II, 4 and 5, and Pl. III, 6). Since the cambium is stimulated rather than killed by the invasion, normal and invaded portions are found functioning side by side and producing normal and abnormal phloem and xylem adjacent to each other (Pl. II, 1). Newly invaded cambium, *i.e.*, that at the edges of the invaded areas, produces phloem and xylem cells showing greater abnormality than those at the center of the areas that have been invaded for some time. This fact, together with the relatively slow rate of invasion of the adjacent normal cambium areas, often results in the formation of a more or less wedge-shape body of gall wood whose edges show greater abnormalities than its center (Fig. 2, E).

Among the abnormalities found in tissues derived from invaded cambium are the following:

1. Increase in radial and tangential diameter of both phloem and xylem elements. In the case of the xylem this often is accompanied by a pronounced shortening of the tracheids, which are often blunt (Fig. 2, D, Pl. II, 4 and Pl. III, 6).

2. An 80 to 100 per cent increase may be shown in the number of xylem cells formed. No comparable increase is shown in the number of phloem elements.

3. Increase in the proportion and number of parenchymatous cells in both phloem and xylem. In the phloem these almost completely replace the sieve tubes (Pl. II, 1), while, in the xylem, the number of parenchymatous cells associated with the resin ducts is so increased that often continuous areas of parenchymatous cells extend from one duct to another, particularly in the areas at the edges of the abnormal wood.

4. Increase in the number of vertical resin ducts in the xylem. This increase, however, is roughly in proportion to the increase in the volume of gall wood, and, though their diameters are abnormally large, many of them are partly or entirely closed through the swelling of their epithelial cells (Pl. II, 5).

5. Frequent production of radial resin passages in the fusiform rays of the phloem, which terminate next to the cortex in resin-containing cavities, somewhat spherical as seen in transverse and in radial longitudinal sections. These have been observed very seldom in normal wood (Fig. 2, D, and Pl. II, 3).

6. Increase in the number, height, width, and cell volume of linear rays in both phloem and xylem. (Fig. 2, D; Pl. III, 4 and 5). The number of xylem rays may be increased by 50 to 60 per cent, as seen in cross section. While the height of normal xylem rays is from 1 to 5 cells and the rays are

comparatively widely separated vertically, abnormal rays vary from 2 to 6 cells high and often appear almost vertically confluent one with another as seen in tangential longitudinal sections (Pl. III, 4 and 5). Width of normal linear rays of the xylem is 1 and very exceptionally 2 cells, while those in the gall wood vary from 1 to 3 and even 4 cells. The much greater volume of the cells in abnormal wood increases the significance of the discrepancies indicated above.

7. Increase in size and number of the fusiform rays comparable to that noted for linear rays.

8. The radial rows of xylem and phloem lose something of their orderly appearance as seen in cross sections (Pl. II, 1), a phenomenon noted in a lesser degree in normal wood making fast growth.

9. Pitting of the tracheids is found on the tangential as well as the radial walls (Pl. III, 5) to which it is considered limited in normal wood of *Pinus sylvestris* (10).

10. Cell walls of abnormal tracheids vary in width but often are twice that of the normal. Walls of the elements produced at the end of the growing season do not differ appreciably in thickness from those of the spring wood and the diameter of the cells is much the same throughout the annual ring, so that these rings are often difficult to distinguish in gall wood.

Discussion of Anatomical Studies

Meinecke (8) and Klebahn (7) have shown that repeating pine rusts may reinfect their pine hosts through the young epidermis of the current season's shoots. In the present study, as in the work of Hutchinson (6), the actual penetration of the epidermis by aecial germ tubes was observed.

While no detailed histological studies have previously been made of galls formed by the Woodgate *Peridermium,* the galls of *Cronartium quercuum* (Berk.) Miyabe (*C. cerebrum*) have received considerable attention. Weir (12) considered the annual growth of the galls to be stimulated by the presence of the perennial mycelium of the fungus in the living sapwood. Dodge and Adams (4) found the mycelium of *C. quercuum* (*C. cerebrum*) in galls on *Pinus rigida* Mill. to be intercellular and uninucleate, abundant in the cortex and apparently following the rays in the phloem and xylem. Haustoria were constricted where they penetrated the cell wall, were found to be abundant in the rays, common in the phloem, and comparatively scarce in the primary cortex.

Stewart (11) found that many of the tracheids in the gall wood of *C. quercuum* (*C. cerebrum*) had blunt ends and were differentiated from the wood parenchyma cells only in their pitting, which, at times, was irregularly 'Araucarian,' with the bars of Sanio seemingly lacking. The pits varied greatly in size and bordering, even in the same tracheid. Nonfusiform rays were often 2 to 3 cells wide, abnormally abundant, and composed of unusually large cells. Three times the usual number of resin canals appeared in cross section, often so close that only the ray cells separated them. Above and below the gall, the normal number was present.

The present study has indicated the similarity of the galls produced by the Woodgate *Peridermium* to those of *C. quercuum* (*C. cerebrum*) as described by Stewart. He, however, interpreted the discolored nonconducting core as resinous in nature, while, in the case of the Woodgate *Peridermium,* the discoloration is probably due to tannin derivatives. Resin was found limited to the canals and did not impregnate the wood in any general fashion. It is well to bear in mind, however, that the term "tannins" as used in this paper includes the broad range of substances that give the "tannin" reaction with ferric chloride. Unlike the galls studied by Stewart, those of the *Peridermium* at Woodgate can, and often do, influence the structure of the host beyond them where no mycelium is present. Beyond the gall, the stem is often of larger diameter than below it, probably because of the fact that the abundance of phloem parenchyma in the gall has cut off much of the normal downward passage of elaborated material, which expresses itself in this diameter increase. With this diameter increase goes an increase in the number of resin canals. At times a tendency toward brooming may arise beyond or on the distal portion of the gall.

EFFECT OF GALL DEVELOPMENT UPON CONDUCTION THROUGH THE XYLEM

On August 18, 1933, conduction experiments were made with eosin to determine in some degree the effect of the gall upon the conduction of water

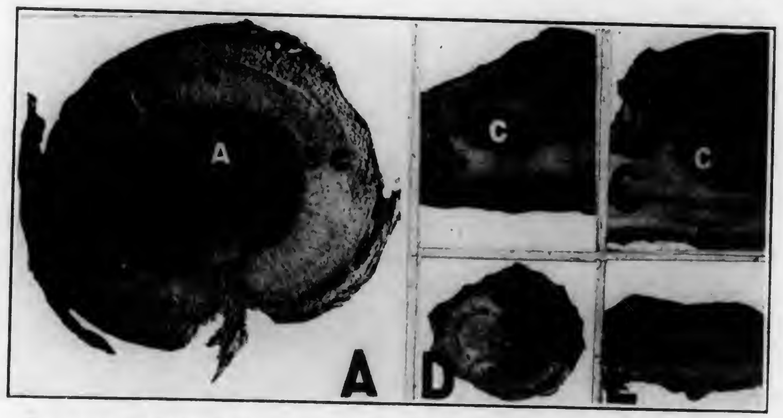

FIG. 4. A. Transverse section through an unstained gall showing dark color of the core (A) and wide area of conducting tissue (B) between it and the cambium at (C). B and C. Longitudinal sections through galls through which eosin has been conducted, staining the conducting tissue as at A and B. Note the contrast between the conducting eosin-stained tissue and the non-conducting naturally discolored core (C) and the nearly complete absence of conducting tissue between the core and the cambium of gall C. The branches beyond A and B appeared normally vigorous while that distal to C was dwarfed and dying. D. Young gall in which whole of gall wood still functioned in conduction and showed the eosin stain. E. Young gall in which the core failed to show the stain as strongly as the outer portion and whose core is inferred to have become at least partially non-conducting, though as yet no natural discoloration had appeared in it.

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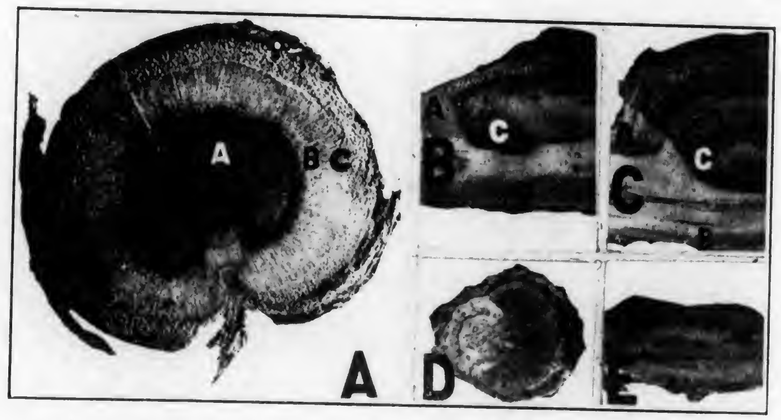

FIG. 4. A. Transverse section through an unstained gall showing dark color of the core (A) and wide area of conducting tissue (B) between it and the cambium at (C). B and C. Longitudinal sections through galls through which eosin has been conducted, staining the conducting tissue as at A and B. Note the contrast between the conducting eosin-stained tissue and the non-conducting naturally discolored core (C) and the nearly complete absence of conducting tissue between the core and the cambium of gall C. The branches beyond A and B appeared normally vigorous while that distal to C was dwarfed and dying. D. Young gall in which whole of gall wood still functioned in conduction and showed the eosin stain. E. Young gall in which the core failed to show the stain as strongly as the outer portion and whose core is inferred to have become at least partially non-conducting, though as yet no natural discoloration had appeared in it.

INTENTIONAL SECOND EXPOSURE

and dissolved substances to the branch beyond. One-, 2-, and 3-year-old portions of stems, with and without galls, were cut off below the surface of an eosin solution. The galls and noninfected areas of the galled twigs and normal twigs were examined when the eosin reached the tips of the current season's wood, and the stained and unstained areas were noted. In this way the presumably water-conducting wood, stained pink, was differentiated from the nonconducting which remained unstained.

FIG. 5. A. Longitudinal section through untreated gall showing discolored core at center. B. Similar section of gall tested for tannins showing the positive tannin reaction of the core. C. Similar section of gall tested for starches showing the positive starch reaction of the woody tissues external to the core and the absence of starch from the core itself.

While all the gall wood of the 1-year-old swellings was active in conduction (Fig. 4, D), an undyed, or lightly stained, central core often appeared in 2-year-old galls (Fig. 4, E). In some of the 2-year-old and all of the 3-year-old galls, the cells of this nonconducting core were filled with brownish substances (Fig. 4, A). These cells and nondiscolored cells close to them contained tannin-like compounds instead of the starches and oils so abundant in the parenchymatous cells of the major part of the nondiscolored gall wood (Fig. 5, A, B, and C). In three-year-old normal stems, however, the whole woody cylinder was stained, and all the tracheids seemed to have been active in conduction.

The nonconducting discolored core of mature galls may be relatively small (Fig. 4, A and B) or may extend outward from the center of the gall and include all the cells of the gall wood except those most recently derived from the cambium (Fig. 4, C). If the first condition exists, the galls may cause little or no abnormality beyond the areas of local swelling. In the second condition, however, the branch beyond the gall may often be dwarfed or dying.

ASH ANALYSES[5]

In the hope that analyses of the ash of galls and rust-free portions of susceptible trees would perhaps indicate some differentiation between the physiological phenomena of gall formation and normal branch development, analyses were made on December 1, 1933, of fresh material from susceptible trees that was divided into two lots. The first contained galls from 2 to 4 years old, and the second consisted of the rust-free portions of the same branches from which the galls were taken. Bark and wood were taken together in both samples. These lots were ashed and analyzed by the method of H. S. Washington.[6]

TABLE 1.—*Results of ash analyses of 2 and 4-year-old galls and rust-free portions of stems of Pinus sylvestris*[a]

Constituent	Rust-free portions		Galls	
	2 Yrs.	4 Yrs.	2 Yrs.	4 Yrs.
CaO	17.76%	26.39%	18.57%	22.88%
MgO	5.38	7.22	7.25	6.50
K_2O	35.03	28.39	30.62	25.90
Na_2O	4.46	2.64	1.58	4.29
N	0.51	0.31	0.70	0.95
SO_3	5.33	4.10	5.15	7.46
P_2O_5	13.09	10.83	19.68	18.59
SiO_2	15.54	11.28	7.99	6.09
Fe_2O_3	2.92	3.37	2.68	2.72
Al_2O_3	0.53	6.10	5.75	5.34
MnO	0.41	0.03	0.18	0.12
Cl	0.2	trace	0.10	trace
% Ash	1.31	0.65	1.38	0.88

[a] Results reported on the basis of a carbon and CO_2 free ash.

[5] The author wishes gratefully to acknowledge the research grant from the University of Pennsylvania Chapter of Sigma Xi, which enabled him to have the analyses made.

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Table 1 shows some divergences between the ash of the galls and the rust-free wood. The greater amounts of nitrogen, sulphur and phosphorus in the galls can perhaps be correlated with the large quantities of food substances stored in them. The lesser amounts of silicon present in the galls appear to add a chemical reason to the structural basis of the mechanical weakness shown by gall wood. Most of the results, however, show differences whose explanation is at present difficult. Perhaps when the complexities of the physiology of the host-parasite relationship are better understood these figures may appear more significant but here the methods of ash analysis appear to shed little light on the physiological aspects of the problem.

RÉSUMÉ OF THE LIFE CYCLE AND MORPHOLOGY OF THE WOODGATE PERIDERMIUM

The Woodgate *Peridermium* is autoecious and has but 2 known spore forms, pycnia and aeciospores. The pycnia were first found during the course of this study. They were discovered in material collected on May 17, which had been selected to show developmental stages of the gall and, in choosing and fixing the material, the pycnia had been passed over unnoted. Later, other inconspicuous internal pycnia were found in sections of material from several young galls collected in the same season some of which had shown external traces of exudate at the time of collection. The aeciospores reinfect the host directly. They are roundly and irregularly rhomboid with verrucose walls. No germ pores have been observed in the walls of the aeciospores. (Fig. 3, D). They may germinate on water or upon the host in from 12 to 24 hours. The germ tube protrudes often but not always from an apex of the spore and broadens to form a septate branching hypha, which, within the inoculation chamber, may apparently penetrate the host at once or grow luxuriantly over the epidermis for a considerable distance before entering.

In most cases the mycelium vegetates within the host for 2 seasons and in May of its third season may produce pycnia from small hyphal wefts beneath the periderm of the central sunken portion of the gall often near its edge (Pl. III, 3). In late May and early June of the fourth season, approximately 36 months after infection and in successive seasons thereafter, aecia are produced. They arise from larger and denser wefts arising deeper in the tissue than the pycnial wefts and presumably radially interior to them, though the exfoliation of the pycnial weft precedes aecia formation. As the aecial weft develops, the host cells within and adjacent to it are separated widely from each other, but often do not appear to die for sometime, in consequence. The peridial cells of the young aecium push up the periderm and the upper portion of the hyphal weft, which may then disappear entirely or remain partially adherent to the edges of the mature aecium. The peridium varies from 1 to 4 cells in thickness (Fig. 6, A and Pl. III, 1) and stalactiform structures of the nature of the peridium are sometimes found uniting the dome of the peridium with the base of the aecium (Pl. III, 2).

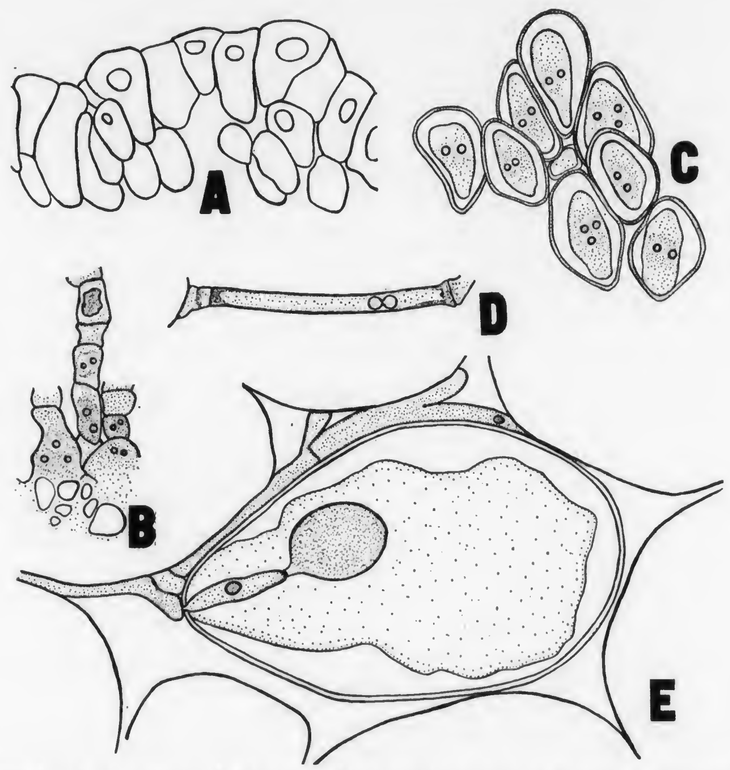

FIG. 6. A. A portion of the dome of the peridium with cells drawn in outline. B. Portion of the bases of aecial spore chains showing binucleate and trinucleate condition. C. Young aeciospores, some binucleate and some trinucleate. D. An apparent binucleate condition in a cell of a vegetative hypha. E. Hyphae and haustorium in the cortex. Note the effects of shrinkage upon the nucleus at its point of contact with the haustorium.

The usual number of nuclei in the vegetative cells and of those making up sterile portions of the wefts is 1, and, while 2 have been exceptionally found in vegetative cells (Fig. 6, D), it seems likely that they do not indicate the initiation of a distinct stage in the life cycle.

A 2-, 3- and, exceptionally, 4-nucleate condition arises in the basal cells of the enlarged hyphae that cut off the young aecial spore chains (Fig. 6, B), and the young aeciospores are in turn usually binucleate but often possess 3 (Fig. 6, C) and at times 4 nuclei. The nuclei in maturing spores apparently become grouped, but no actual fusion has been observed. The young germ tubes, however, often appear to have 2 or more nuclei per cell, but only 1 has been observed in each cell at the time of host penetration. Until further cytological studies can be made upon this organism, the interpretation of these nuclear phenomena would appear hypothetical.

Dodge and Adams (4) found that of a collection of *Cronartium quercuum* (*C. cerebrum*) galls on *Pinus rigida* from New Jersey, some produced only

aecia and others only pycnia per season, and concluded that aecia and pycnia probably were produced on these galls in alternate years. In the case of galls on *P. virginiana* Mill., however, both aecia and pycnia developed on different parts of the same gall in the same year. While galls of repeating western rusts may produce aecia annually, Gill (5) reports that their pycnia are exceedingly rare.

GENERAL DISCUSSION

The Woodgate *Peridermium* is known to be definitely autoecious at Woodgate, New York.[7] The fact that its aecia produce germ tubes that reinfect the host without the production of sporidia precludes the possibility that we have here to do with the microtelia of a typical microcyclic rust. This *Peridermium*, then, in the character of its life cycle, seems only comparable to the two western repeating forms studied by Meinecke (8) and referred to by him as *Cronartium harknessii* Meinecke and *Peridermium cerebroides* Meinecke, and now grouped together by Arthur (2) as forms of *C. coleosporioides* (D. and H.) Arth. and to *P. pini* (Willd.) Kleb., a European repeating pine rust that does not form galls studied by Klebahn (7). The Woodgate *Peridermium* seems, however, to produce pycnia with greater frequency than either of the western forms, and its peridium, 1 to 4 cells in thickness, contrasts markedly with that of the repeating aecial form of *C. coleosporioides* (*C. harknessii*) whose peridium is 5 to 7 cells in thickness (1).

Intracellular penetration was found more frequently than penetration between epidermal cells. The early intracellular development of the mycelium in the epidermis and its formation of atypical, branching, septate haustoria in the subepidermal cells suggest the findings of Pady (9), who has shown the early tendency of infections toward intracellular development to be even more pronounced in the short-cycle orange rust of *Rubus*.

The coincidence of the paths of early, unresisted cortical invasion with the regions more richly supplied with starches and oils seems to signify that there may be a relationship between the amounts of food available to the rust and the supply of demonstrable starches and oils. It is interesting to note that the tannin-like compounds, present in normal quantities in the vacuoles of many cells in these regions, do not interfere with the migration of the mycelium along these paths.

Bearing in mind the possible relationship of potassium to foods available to the rust, Hutchinson (6) considered that resistance varied with the host and found that trees whose ash contained little potassium were more susceptible than those with larger amounts of it. He considered that infections that fail to produce galls in susceptible trees did so primarily through competition and lack of nutriment. In this paper the view is taken that, at Woodgate, susceptibility varies not only with the host but also with the parasite, and that there is a question of compatibility involved between the host and each mycelium whose germ tube penetrates it. It may well be borne in mind, too, that the entrance of secondary organisms, some of which have

[7] H. H. York. The Woodgate *Peridermium*. [Manuscript.]

quite frequently been noted in histological preparations of young galls, might influence the reaction of otherwise compatible hosts.

It is true, as Hutchinson has pointed out, that different parts of the same tree may produce galls more readily than others following infection. The host tissue of a single shoot is a variable factor, since portions of the young stem, such as areas at the needle bases, may be better supplied with starches, oils, etc., than others. Yet it seems unlikely that a commandeering of the food supply by the first mycelium to penetrate could explain the wide variation of host reaction to the different invading mycelia in inoculated twigs where the infections may be considered to have taken place at about the same time. In these cases, too, as in others studied, there seems to have been no relation between the compatibility of the mycelium with its host and the region of the shoot attacked, though definite regions of abundance and scarcity of demonstrable stored foods do exist in the shoot as indicated. Also, areas invaded by incompatible mycelia and those adjacent to them, rather than being free of demonstrable foods, are abnormally full of starch and oil in the early stages of invasion.

Compatibility in susceptible trees, then, seems to vary not only with the host but with some property of the infecting mycelium. That this variable property is a physiological one seems clear. It appears likewise unlikely that a variation in vigor of the invading mycelium is the sole cause of the variation in the host reaction. Rather, it seems likely that some mycelia are genetically better adapted than others to achieve and maintain the metabolic balance with the host so necessary to obligate parasites. Perhaps the varying number of nuclei in the aeciospores may indicate such an instability in the genetic constitution of the *Peridermium* at Woodgate. The question is here raised whether we do not, then, have in this case of a forest tree rust something comparable to the physiologic races recognized among cereal rusts.

Even in the cases of most complete compatibility, the cells of all the invaded areas except the cambium eventually die and, in contrast to phenomena sometimes exhibited in the cereal rusts, die sooner than cells of comparable but uninvaded tissues. This is true of the invaded cortical tissues as well as of the phloem and xylem. The length of time intervening between invasion and death of the cortical tissues usually measures the compatibility of the reacting organisms and consequent susceptibility of the host, since an infection that reaches the cambium seldom fails to cause gall formation. The more compatible mycelia at first cause but little abnormality in the structure or contents of invaded parenchymatous host tissues, but gradually these become filled with starches and oils and, just previous to death of the cells, unusually large amounts of tannins appear in them in the place of the oils and starches. Where compatibility is less, this series of events follows in swifter succession. Coincident with, or shortly after the appearance of an abnormal abundance of tannins in cortical cells, less compatible mycelia meet the resistance of hypertrophy or of hyperplasia or both and the degree of structural abnormality of the tissues is proportional to the physiological

abnormality expressed in terms of host cell contents. Coincident with the death of the cells in the invaded area, a row of meristematic cells arises to form a cicatrix, which proves to be a barrier to invasion only when death of the cells occurs so quickly after invasion that the mycelium has no time to grow beyond the zone of cicatrization.

Only cambial initials in close proximity to the hyphae are influenced by the presence of the organism to produce abnormal phloem and xylem elements. Parenchymatous cells are produced in much greater proportions than normal and these are soon filled with abnormally large amounts of starches, oils, and, in the case of the phloem parenchyma, tannins. Thus, the parasite appears to cause its host not only to provide it with extra food but also with extra food-containing cells. These abnormalities are most pronounced in tissues derived from newly invaded cambium, and there is some suggestion of a return toward the normal in tissues arising from initials in cambium areas that have been invaded for a year or more. After periods of time, which vary in different mature galls from less than 1 to as much as 3 years after their formation, invaded tracheary xylem elements cease to conduct, the starches and oils are replaced by tannins, some of which seem to be located at the periphery of the cells, the cells die, and the mycelium in the affected area dies with them. When death occurs too soon after cell formation, *i.e.*, too close to the cambium, so that the number of conducting cells is much below normal, the branch beyond the gall often shows dwarfing and may die probably, at least in part, because of water shortage. The distance between the cambium and this nonconducting core may be a further measure of compatibility.

SUMMARY

The life cycle of the Woodgate *Peridermium* has been followed histologically from penetration of the epidermis of the current season's shoot of *Pinus sylvestris* by aecial germ tubes through gall formation to production of pycnia 2 years after penetration and repeated formation of aecia in ensuing seasons.

The wide variety of early response of single susceptible twigs to invasion suggests the existence of more than one physiologic race of the *Peridermium* at Woodgate.

Mycelia causing little early abnormality in the tissues invaded are considered as compatible with the host. These penetrate the cortex more swiftly along characteristic diverging paths found to possess demonstrable nutrients in greater abundance than areas traversed more slowly.

In the case of mycelia extremely incompatible with the hosts, starches, fatty substances, and, finally, tannin-like compounds are rapidly concentrated in cells near the hyphae. Following the deposition of abnormally large quantities of the last, these cells die and the fungus dies with them.

The more compatible mycelia grow out in advance of this killing and succeed in forming galls, but cell hypertrophy, hyperplasia, and barrier formation often hinder their passage through the cortex.

Compatible mycelia invade the matured secondary tissues by the end of the first season, while others may be delayed. Phloem and xylem are invaded mostly along medullary rays. Stelar tissues, matured before invasion, show comparatively slight structural change, but their parenchymatous cells become abnormally full of starches, fatty substances, and tannins.

The presence of the fungus in the cambium causes the production of abnormal secondary tissues containing a high proportion of parenchymatous cells that become in turn filled with starches, fatty substances, and, finally, tannins. Increase in cell size characterizes both phloem and xylem, while only the latter shows a marked increase in cell number. Irregularities in size, shape, and pitting of the tracheids also are produced.

Portions of the cambium penetrated for the first time produce the most abnormal secondary tissues, so that areas of gall wood are outlined by tissues showing the greatest abnormalities.

Abnormal parenchyma tissues in the xylem at first contain large amounts of starch and fatty substances, which are replaced by tannin-like compounds usually during the third season in the oldest gall wood, which then ceases to conduct. In cases where the nonconducting core occupies nearly all of the xylem, the branch beyond the gall often dies.

Meanwhile, the death of the inner cortex and of the abnormal phloem cells leaves portions of the cortex external to them isolated from radial conduction. These portions die, collapse, and later are exfoliated except at the edges, where, by contrast, they appear as a raised collar.

Early in May 2-year-old galls often bear pycnia hidden directly beneath the periderm below the sunken central area. This is the first report of pycnia for the Woodgate *Peridermium*.

Three-year-old galls often produce confluent aecia from extensive dense mycelial wefts in the sunken area arising slightly below those of the then exfoliated pycnia. Each year the tissues containing the aecial weft are exfoliated by a periderm arising internal to them and the next year aecia are formed below the new periderm.

Ash analyses of 2- and 4-year-old galls and rust-free portions of susceptible trees showed the galls to be significantly lower in silicon and higher in nitrogen, phosphorus, and sulphur. Other discrepancies were slight or difficult to interpret.

Preliminary nuclear studies revealed the perennial vegetative mycelium to be predominantly uninucleate. Two, 3 and occasionally 4 nuclei appear in the cells at the base of the aecial chain, the young aeciospores, and many portions of the germ tubes. The latter, however, are markedly uninucleate when they penetrate the host.

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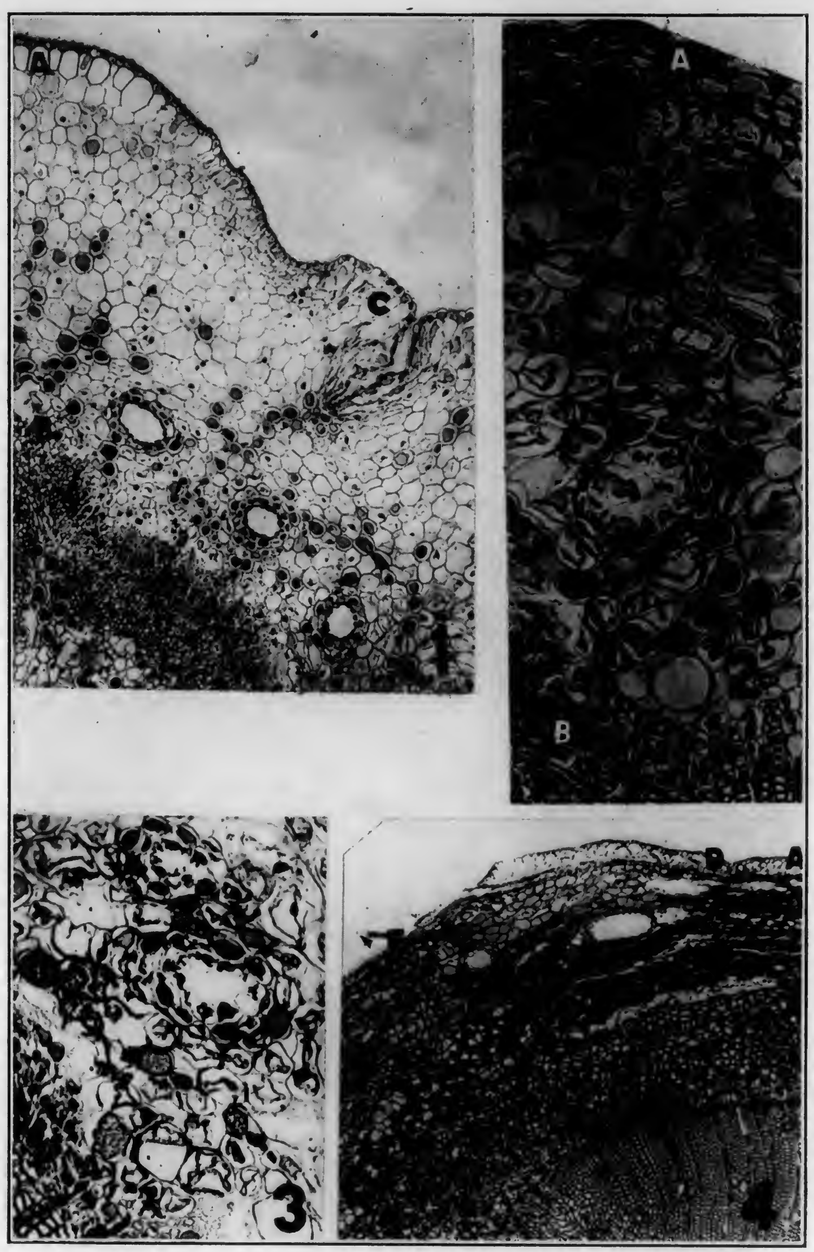

PLATE I

1. Transverse section of a normal twig of *P. sylvestris* as it appears in early June. Note especially the thickness of the walls of epidermal and subepidermal cells at A, the base of the needle fascicle supplied by the bundle trace at B as contrasted with the thinner walls elsewhere as at C. 2–3. Transverse sections showing unresisted invasions. 2. shows a very limited necrosis at point of penetration (A), scarcity of hyphae in the outer cortical region as compared with the inner, especially in the vicinity of the resin canal and the closing of the canal by the hypertrophy of the epithelial cells. The mycelium is shown entering the stelar tissues through a bundle trace at B. 3 shows a resin canal still open in spite of the abundance of hyphae near-by. The mycelium enters the phloem of the main stele at A. 4. Early stage in the formation of the "collar". Necrosis has spread from near the center of infection (A) largely in the inner cortex to B and this, together with pronounced abnormalities arising in the inner cortex and phloem from B to C, has partially isolated the outer cortex external to it. Following its isolation, it has become severely necrotic from A to D and appears to be dying also from D to E where a barrier from D to C is forming to separate the dying portion from the more normal cortex at the left in the photograph.

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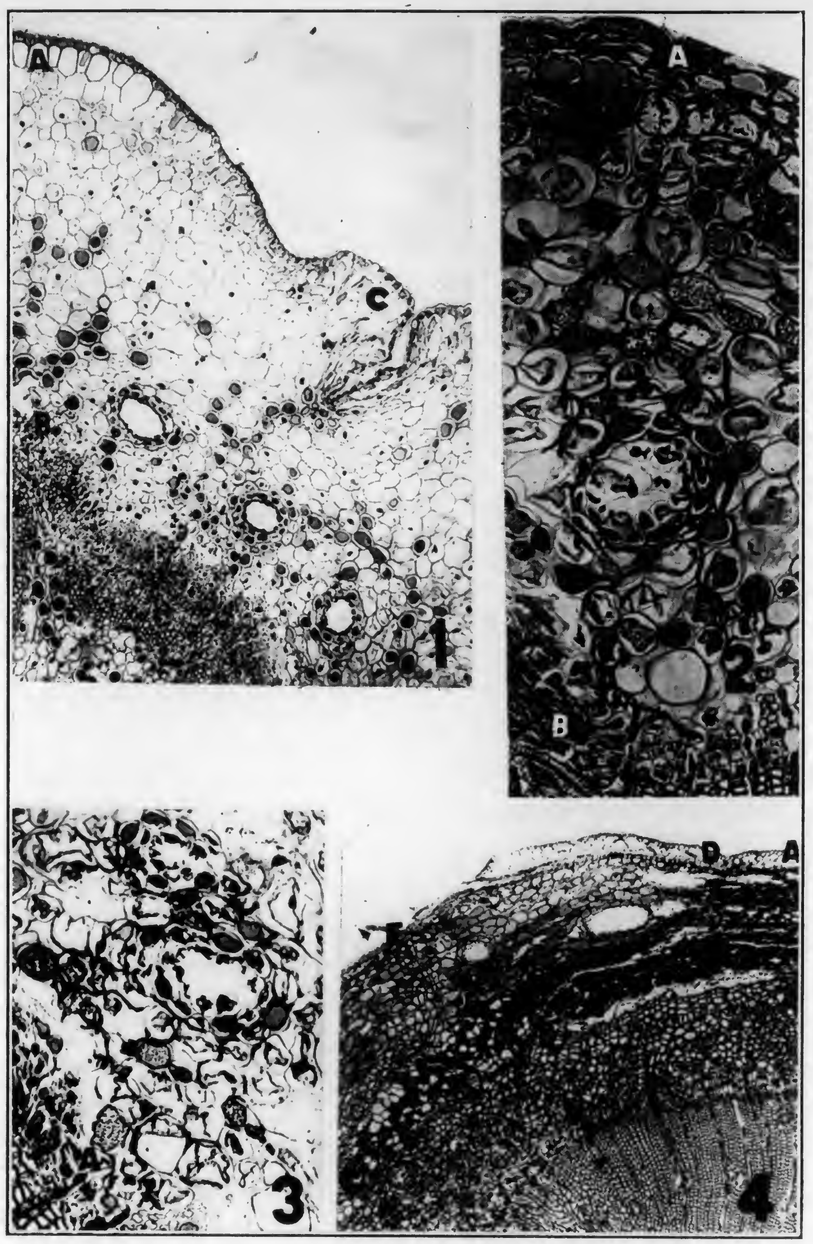

PLATE I

1. Transverse section of a normal twig of *P. sylvestris* as it appears in early June. Note especially the thickness of the walls of epidermal and subepidermal cells at A, the base of the needle fascicle supplied by the bundle trace at B as contrasted with the thinner walls elsewhere as at C. 2–3. Transverse sections showing unresisted invasions. 2. shows a very limited necrosis at point of penetration (A), scarcity of hyphae in the outer cortical region as compared with the inner, especially in the vicinity of the resin canal and the closing of the canal by the hypertrophy of the epithelial cells. The mycelium is shown entering the stelar tissues through a bundle trace at B. 3 shows a resin canal still open in spite of the abundance of hyphae near-by. The mycelium enters the phloem of the main stele at A. 4. Early stage in the formation of the "collar". Necrosis has spread from near the center of infection (A) largely in the inner cortex to B and this, together with pronounced abnormalities arising in the inner cortex and phloem from B to C, has partially isolated the outer cortex external to it. Following its isolation, it has become severely necrotic from A to D and appears to be dying also from D to E where a barrier from D to C is forming to separate the dying portion from the more normal cortex at the left in the photograph.

INTENTIONAL SECOND EXPOSURE

PLATE II

1. Transverse section showing the edge of an infection with invaded and uninvaded secondary tissues side by side. Note that in the pathic phloem the parenchyma cells with their deeply staining contents (A) have almost completely displaced the sieve tubes (B), which comprise most of the phloem in uninvaded tissues. 2. A cicatrizing zone formed from A to B to C to D has proved ineffectual in resisting the invasion as has the similar zone from A to E to F, which formed later. Note that the mycelium is already present in the phloem G and xylem H. Radial resin duct in uninvaded tissue uniting the resin cavity A in the phloem with the vertical xylem resin duct. 4. Radial longitudinal section showing edge of infection where the mycelium is advancing vertically and radially into uninvaded phloem (A) and crossing the cambium to enter the very abnormal xylem. Note that abnormalities in the secondary tissues are produced at some distance in advance of the mycelium. 5. Transverse section showing mycelium crossing the cambium and distributed mostly radially in pathic phloem and xylem though not always confined to the rays.

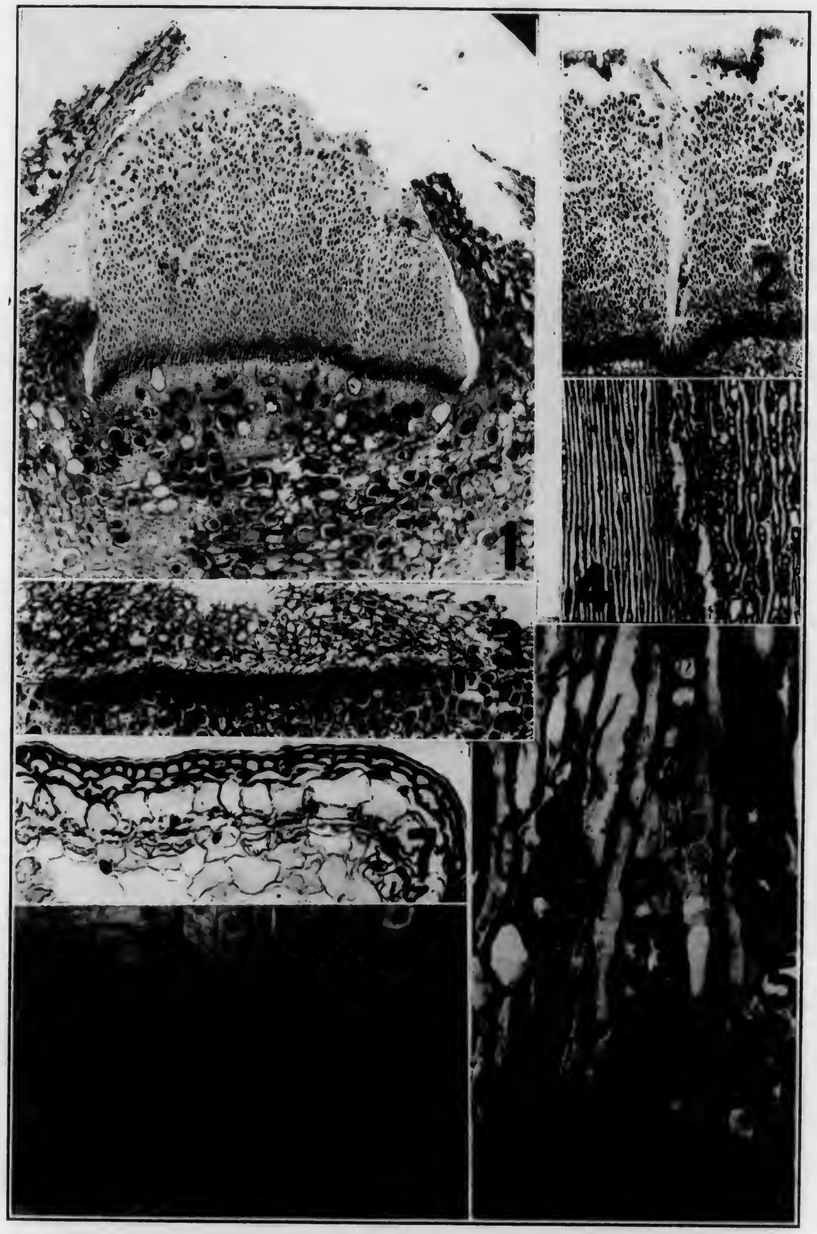

PLATE III

1. Section through a small aecium showing external periderm, the peridium of the aecium, the darkly stained basal cells of the spore chains, and the widely separated host cells below the aecium. 2. Section through the filaments occasionally found showing their double character and suggesting that they are separations between otherwise confluent aecia. 3. Section through a pycnium showing the very limited pycnial weft and its position below the periderm. 4. An approximately tangential section through gall wood and uninvaded wood adjacent showing the irregularities and large size of the cells of the gall wood and their disproportionately large amount of parenchymatous tissue. 5. A higher magnification of a portion of the gall wood shown in 4, showing particularly the tangential pitting of one of the walls of one of the tracheids at A. 6. Radial longitudinal section showing the mycelium oriented radially in and near the cambium. 7. Epidermis and periderm of normal *P. sylvestris* as they appear in early August.

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INTENTIONAL SECOND EXPOSURE

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THE PARASITISM OF POLYPORUS SCHWEINITZII ON SEEDLING PINUS STROBUS[1]

ROBERT E. WEAN[2]

(Accepted for publication August 24, 1937)

INTRODUCTION

Polyporus schweinitzii Fries, as a cause of root rot in coniferous trees, was reported by Sargent (23), in 1897, to be more destructive in the United States than in Europe. Von Schrenk (25), in 1900, stated that this organism was very common in our northern forests of spruce and fir. In 1933, York[3] discovered *P. schweinitzii* causing root and root-crown decay in forest plantings of white pine, *Pinus strobus* L., established in 1912–1914.

It is generally believed that this fungus attacks mature and over-mature coniferous trees by invasion of the roots through wounds. The penetration of root parasites into woody plant tissue has been extensively studied by several investigators (6, 11, 18, 25, 29). However, our knowledge pertaining to *Polyporus schweinitzii* is based entirely upon field observations. From this information it appears to have a wide range of coniferous hosts (2, 8, 12, 13, 21, 25, 34). It also has been reported on hardwood trees (15, 22). The writer is not aware of any published record of the ability of this fungus to enter its host directly or of infecting and injuring the living roots of seedling conifers.

York's conclusion (*op. cit.*) that this organism is, apparently, a direct parasite and that there seems to be a close relation between the amount of infection and the pH of the soil suggested this investigation. The influence of temperature, hydrogen-ion concentration, and mineral nutrition on the

[1] A dissertation in botany, presented to the Faculty of the Graduate School, University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to acknowledge the advice and criticism of Dr. H. H. York during the progress of the work and the facilitation of chemical analyses indicated. Appreciation is expressed to Mrs. Minnie Taylor York, Dr. Conway Zirkle, and Dr. W. G. Hutchinson for their suggestions regarding the manuscript.

[3] York, H. H. A study of a resinosis and root rot in forest plantings of *Pinus strobus* L. and *P. resinosa* Ait. [Manuscript.]

susceptibility of various plants to attack from fungous parasites has been demonstrated by numerous workers (3, 7, 9, 16, 27). Since *Polyporus schweinitzii* is now known to be exceedingly destructive in the Rochester watershed plantings of northern white pine in the State of New York, young trees of that species were utilized in this study in order to determine whether such factors as soil acidity and nutrition influenced the susceptibility of the host. A study of the physiology of the fungus also was attempted with a view to a more complete interpretation of host reaction.

MATERIALS AND METHODS

The original isolation of *Polyporus schweinitzii* used in this study was made from tissue of a sporophore collected from a severely infected white-pine plantation near Hemlock Lake in the state of New York. The inoculum was prepared by filling Petri plates two-thirds full of crushed white-pine needle duff to which was added sufficient 2 per cent Trommer's plain malt solution to secure a saturated condition. Having been autoclaved, they were inoculated at the center of the dish and held at room temperature in diffused light. The medium was ready for use when the fungus showed good growth over and through the duff.

The determination of the parasitism of young trees by this fungus involved the following procedure: 1. The use of seedlings immediately following germination of seed, and 2-year-old trees from a nursery. 2. Plants grown under 5 different nutritive conditions in the greenhouse, *i.e.*, a complete nutrient solution adjusted to the pH points of 4.5, 6.0, 7.0, and 2 conditions of nutrient deficiencies at pH 4.5. 3. For each of the 5 conditions 12 pots composed a series; 8 of these pots were inoculated with *Polyporus schweinitzii,* and 4 served as controls, the latter having been added non-inoculated duff. In such an arrangement there were 60 pots of young seedlings and 60 of 2-year-old trees, each nutrient series being thus run in duplicate with the exception of the difference in age of the trees. Pot cultures of quartz sand, to which the nutrient solutions were supplied by the drip method, were deemed advisable for this study. The sand, after being thoroughly washed in running water, was placed in 6-inch clay pots for the young seedlings and 8-inch pots for the 2-year-old trees.

The white-pine seed used in this study was collected in 1933 in the State of New York. Prior to being placed on sterile sand in large moist chambers for germination, they were scarified with sand and were then surface-sterilized with 1–1000 bichloride of mercury for 2 minutes, and washed twice in 2 washings of sterile distilled water. A half plate of the medium was placed at a depth of 1 inch below the surface of the sand before planting 12 germinated seed in each container.

The 2-year-old seedlings were obtained from the Saratoga Nursery of the New York State Conservation Department. They were "lifted" from

IRREGULAR PAGINATION

TABLE 1.—*Growth and infection data of young seedlings*[a]

Series	Nutrient	Initial pH of nutrient	Shoots				Roots					
			Needles		Stem							
			Length	pH of extract	Length	pH of extract	Per cent infected	Number of laterals	Tap root	Total root system	pH of extract
			mm.		*mm.*				*mm.*	*mm.*	
A[b]	Complete	4.50	21.2	5.20	69.4	5.60	20	14.9	109.5	236	5.00
A–e	"	"	21.2	5.25	70.0	5.60		16.3	112.0	275	4.96
B	"	6.00	19.5	5.26	64.6	5.63	37	20.7	80.7	204	4.91
B–e	"	"	22.3	5.15	66.0	5.50		22.9	88.9	308	5.15
C	"	7.00	25.3	5.00	66.5	5.60	52	23.5	95.3	400	5.37
C–e	"	"	25.4	5.27	73.8	6.12		27.2	92.5	422	5.82
D	Low nitrogen	4.50	27.6	4.85	70.3	6.10	28	17.5	81.0	237	5.20
D–e	"	"	27.1	4.95	72.3	5.65		20.0	115.0	302	5.30
E	Low phosphorus	4.50	24.7	4.70	59.8	5.30	52	14.4	44.8	184	5.00
E–e	"	"	24.5	4.70	61.0	5.60		16.1	81.5	208	5.15

[a] Average of 10 plants.
[b] Series A, B, C, D, and E—inoculated; A–e, etc.,—controls, noninoculated.

the seed beds in the fall of 1934, shipped to the University of Pennsylvania, potted in soil, and held over the winter in a cool greenhouse. On March 1, 1935, just as new growth was beginning, the plants were carefully removed from the soil and all foreign material washed off of the roots. Two plants were placed in each 8-inch sand culture. In this case a Petri plate of the needle-duff medium was put directly in contact with the root systems when the plants were potted.

Since few facts were available on the nutrition of white pine in sand cultures, the search for a satisfactory nutrient constituted a problem in itself, for it was necessary to provide one whose acidity might be altered over a wide range. The basic nutrient decided upon, following a personal communication with Dr. J. W. Shive[4] consisted of the following: $Ca(NO_3)_2$ 5.0 cc.; KH_2PO_4 2.5; $MgSO_4$.$7H_2O$ 2.5; $(NH_4)_2SO_4$ 1.5; $Fe_2(C_4H_4O_6)_3$ 1.0; H_2O 1000.0.

The salts were made up as molar solutions with the exception of the ferric tartrate of 1 per cent concentration. A nutrient solution composed of the above elements was used for Series A, B, and C after adjustment to pH 4.5, 6.0, and 7.0, respectively, by the addition of 0.1 N NaOH (Table 1). Two series with a reduction in the amount of an essential element completed the arrangement; namely, Series D in which the NO_3 ions were partially replaced by Cl ions through the reduction of $Ca(NO_3)_2$ to 1.25 cc. and the addition of 3.75 cc. of $CaCl_2$; Series E in which the PO_4 ions were partially replaced by Cl ions through the reduction of KH_2PO_4 to 0.625 cc. and the addition of 1.875 cc. of KCl. The two latter series were adjusted to pH 4.5.

The methods utilized, whereby the nutrient might drip continuously into the pot-cultures, were of two types. For the young-seedling experiment the technique of Shive and Stahl (28), as modified by Trelease and Thomson (30), was followed. Because of the time and labor of maintenance in an experiment of this size, a different system was devised for the series containing the older plants. This system, as previously described by the author (33), consists essentially of a central reservoir for each series, an automatic flow meter, and a main feed line with a capillary tube leading to each pot.

The plants were maintained in culture for 220 days. During this time, root isolations were made for the organism and data upon the plant reactions were obtained. At the end of the period, the plants were carefully lifted from the sand, washed, and the growth and infection data recorded.

The "typical plant," as outlined in Table 1, is a result of an average for 10 plants selected at random in the control and inoculated pots of each series. In the study of the 2-year-old plants (Table 2), 8 trees from the inoculated pots and 4 from the controls, selected as above, were carefully

[4] Shive, J. W. New Jersey Experiment Station, New Brunswick, New Jersey.

TABLE 2.—*Data showing the average growth of the 2-year-old trees during the experiment*[a]

Series	Nutrient	Initial pH of nutrient	Shoots			Roots	
			Needle length	Stem length	New growth	Number of roots	Total new root growth
			mm.	*mm.*	*mm.*		*mm.*
A[b]	Complete	4.50	60.0	151	166	265	2116
A–c	"	"	81.0	156	200	239	2264
B	"	6.00	68.3	109	142	200	1614
B–c	"	"	70.5	139	215	305	2497
C	"	7.00	74.3	106	106	374	2251
C–c	"	"	75.1	153	171	441	3746
D	Low	4.50	77.9	125	161	163	1915
D–c	nitrogen	"	80.0	140	183	218	2541
E	Low	4.50	65.6	148	155	161	2068
E–c	phosphorus	"	81.9	144	189	277	2692

[a] Based on 8 inoculated and 4 control plants for each series selected at random.
[b] Series A, B, C, D, and E—inoculated plants; A–c, etc.,—controls, noninoculated.

examined in each series. The extract for pH determinations was secured by grinding the plant tissue with damp quartz sand in a mortar. To the extract was added 10 cc. of distilled water prior to making the readings electrometrically with a quinhydrone electrode. Root material was fixed for histological study in a saturated aqueous solution of salicylic acid plus chromic sulphate and formaldehyde (4). Representative plants for each series were reserved for photographic purposes and ash analyses.

Since reddish areas have been reported occurring in roots infected by *Polyporus schweinitzii*, an injection experiment was made in an attempt to determine whether a fungous extract would produce a similar change in woody tissue of young white-pine trees. The extract was made by grinding fungus mats with sand in a mortar and filtering the liquid, plus distilled water, through a Seitz filter. This extract was introduced by capillary injection into the stems of 3-year-old trees. In similar manner sterile-needle punctures and injections of distilled water were made in control trees. After a period of 3 months, sections from plants thus treated were made for microscopic examination.

In order to determine whether during growth the fungus altered the pH of a medium, duplicate liquid cultures of surface and submerged type were arranged over a pH scale of 2.59 to 7.0. The nutrient consisted of a 2-per cent Trommer's plain malt solution adjusted to the various pH points by the addition of hydrochloric acid and sodium hydroxide.

For the purpose of determining whether an acid was produced by this fungus during growth, culture solutions, buffered by potassium hydrogen phosphate and citric acid, were arranged over a pH scale of 2.0 to 8.0. All cultures of the fungus were held at room temperature and secluded from

direct light. Examination for changes in the medium was made after 14 days.

RESULTS

Pot Cultures of Young Seedlings. The plants in the control cultures showed that distinctive growth reactions occurred when young white-pine seedlings were grown under various nutritional conditions. Physiologically, the only variables for the different series were introduced by varying the pH and the mineral content of the nutrient solutions. The character of growth response for each series and the results from inoculations with the fungus are shown in table 1.

The apparently normal seedling plants in the control cultures of Series A, receiving the full nutrient at pH 4.5, developed sturdy stems and bright green needles, and their light brown roots bore many root hairs (Fig. 1, A). The response of the inoculated plants in this series was not strikingly different from that of the controls. Root and shoot development was not seriously impaired, although 20 per cent of the lateral roots were infected. In a few cases (Fig. 1, A) the plants sustained severe root injury. The infection of the roots was characterized by localized brownish areas, and the portion toward the root tip ultimately died.

The control plants of Series B (Fig. 1, B), with the full nutrient adjusted to pH 6.0, were apparently less healthy than those of Series A. While the shoot growth was about equal, the needles were a paler green. The root system of the plants in Series B was more extensive and bore more laterals with well-developed root hairs. The inoculated seedlings of this series were more heavily infected than those of Series A, since infection occurred on 37 per cent of the roots. Root and shoot formation was noticeably less than that of the controls; death of the tap root was characteristic of these plants (Fig. 1, B).

The seedlings of Series C, grown at a pH of 7.0, differed decidedly from those of Series A and B (Fig. 1, C). The needles, although well developed, were a pronounced pale yellow-green. Although the stem growth was surprisingly vigorous, the root system was dark brown, and consisted of many fine, short laterals. Root hairs were short and sparse. The inoculated plants showed a higher degree of susceptibility, as infection occurred on 52 per cent of the roots. The roots were near the surface of the sand (Fig. 1, C), as in the controls, thus penetration of the root system was not noticeably altered. Although the number of lateral roots increased with the adjustment of the nutrient solution toward pH 7.0, the number of infected roots increased in still greater ratio.

With a reduced supply of available nitrate nitrogen, the seedlings of Series D (Fig. 1, D) appeared, during the first 4 weeks of growth, to develop in height at a greater rate than those of the other series. This agrees

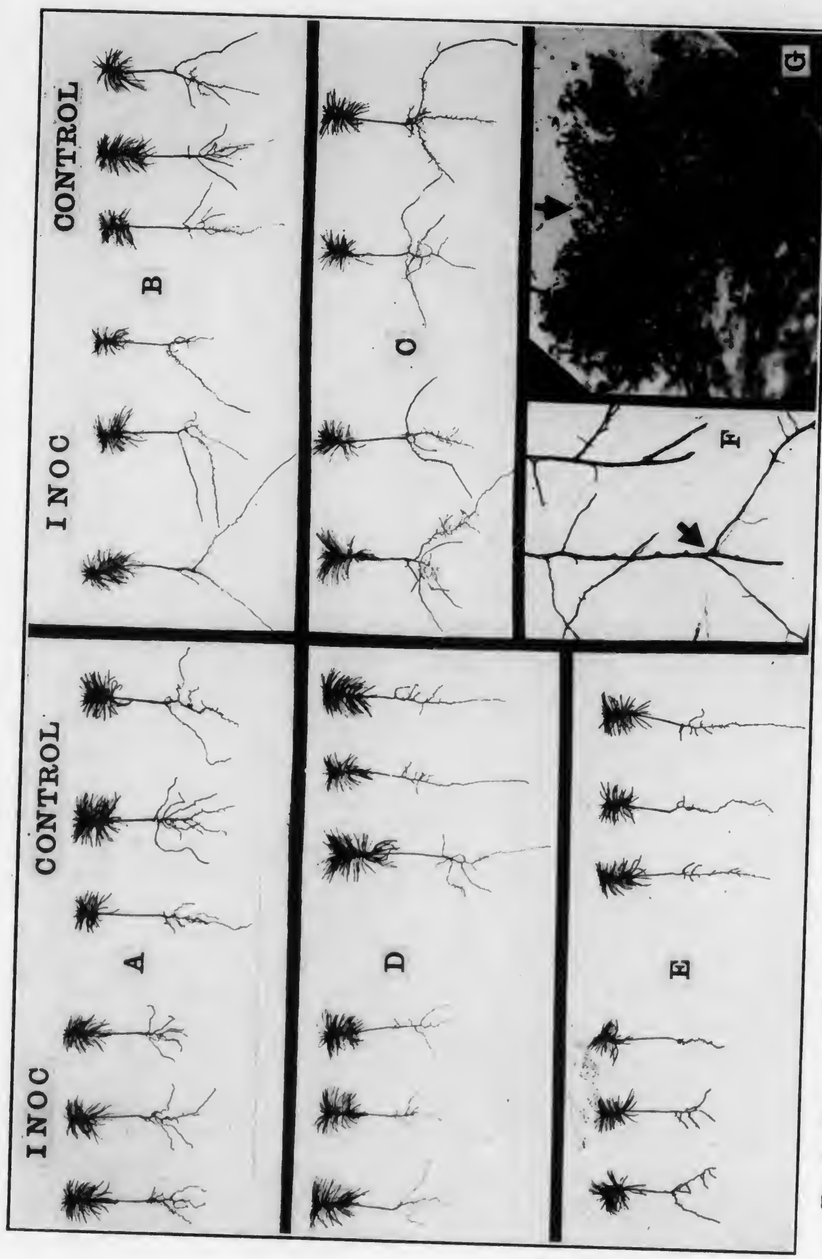


FIG. 1. Young seedling plants of control and inoculated cultures. ×½. Series A, D, and E grown at pH 4.5: A, receiving the full nutrient; D, low nitrogen; and E, low phosphorus. Series B and C with plants receiving the full nutrient at pH 6.0 and 7.0, respectively. The control plants illustrate clearly the effect of changes in the nutrient solution on root development. Inoculated plants illustrate the typical taproot destruction on young plants, and the reduction in length and number of lateral roots by the parasitism of the fungus. F. Corky exerescences arising from the cork phellogen on the root systems, especially at the base of the lateral roots. G. Enlarged section of F showing the loose cell structure. ×40.

with the growth of Corsican pine as recorded by Aldrich-Blake (1). This shoot reaction, however, was barely perceptible by the end of the experimental period. The diameter of the stems was slightly less than that of those receiving the full nutrient and the needles of a slightly paler green. The amount of root injury in the inoculated cultures, 28 per cent, was greater than under conditions of higher nitrogen concentration, and may be interpreted as showing that nitrogen deficiency may induce an increased susceptibility.

The reduced supply of phosphorus greatly retarded the growth of roots and shoots in the controls of Series E (Fig. 1, E). The needles, although of a deep green, were of normal length. Fifty-two per cent of the roots of inoculated plants were infected, which is identical with that occurring under conditions of a full nutrient at pH 7.0.

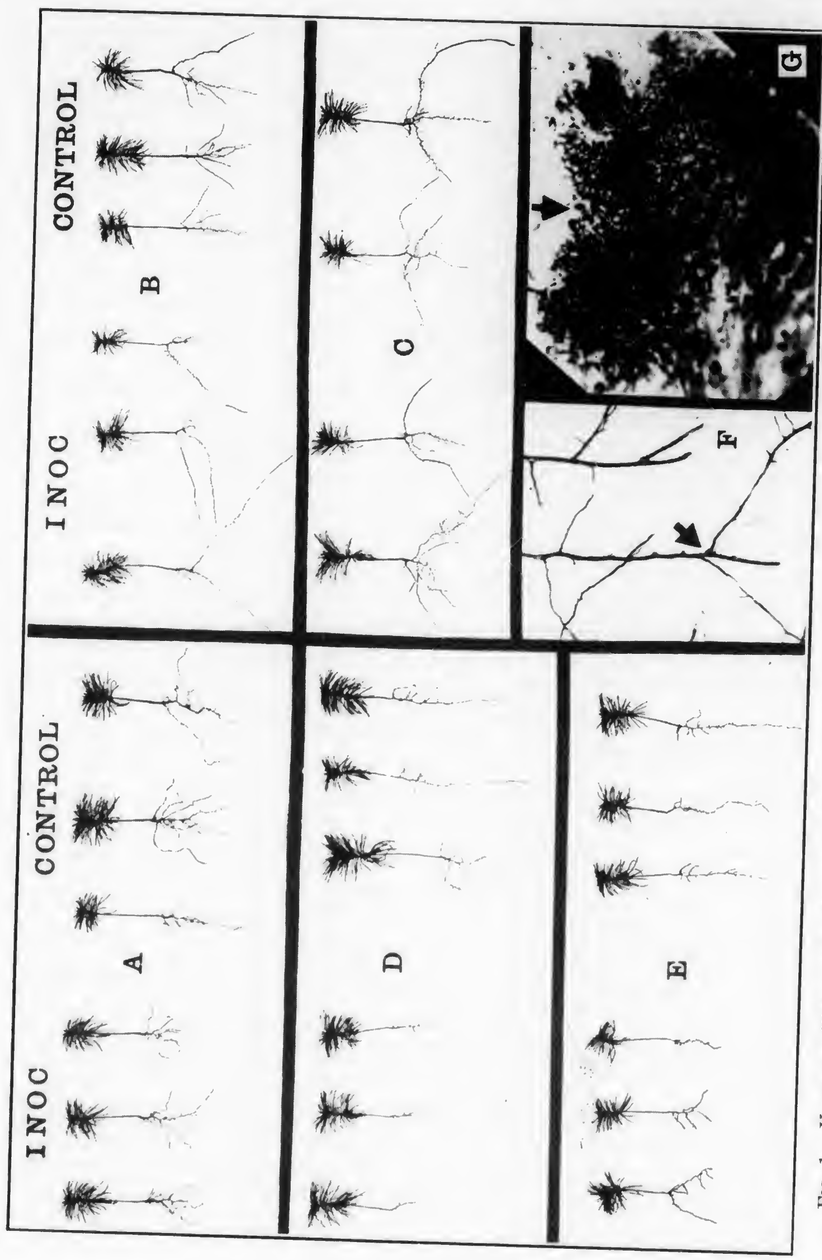
Pot Cultures of 2-Year-Old Trees. In many respects the older plants responded in a manner similar to that of the young seedlings in the different series. The control plants of Series A showed good shoot development, and the character of the root systems indicated favorable growing conditions. This was the only instance in which the number of new roots per plant in the inoculated cultures exceeded that of the controls (Table 2). From table 3 it is plainly evident that infection by the fungus was not severe on roots of the current season or on those of greater age.

The fact that the needles of the control plants at pH 6.0, Series B, were slow in assuming a normal green indicated less favorable growing conditions than those described above. Shoot growth was little affected except in color. There was an increase in number of roots and in the extension of the root system. Infection in this series was apparent on roots of both the current and previous seasons. Open lesions of decay were of common occurrence.

The poor color of new shoot growth at pH 7.0, Series C, was very marked; in fact, the color did not become a healthy green during the entire experimental period. The lateral roots were greatest in number and in total length of the 5 series, but they were very slender and a dark brown. The infection was severe (Fig. 2, C). The percentage of infected roots of the current season's growth was more than twice that of Series B and 5 times that of Series A. In the roots of previous seasons' growth, the percentage dead was nearly 3 times that of B and 6 times that of Series A (Table 3).

In Series D, at pH 4.5, low nitrogen appeared to be a factor affecting the growth of trees at this age, for shoot growth was less than that of Series A. The number of roots also was less, but the root system was of greater total length. The per cent of dead and infected roots of the current season and of the older roots was similar to that of Series B, but the development of lesions was less pronounced.

Under conditions of reduced phosphorus, shoot growth was again less than that of the full nutrient series at pH 4.5. The root systems were char-



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INTENTIONAL SECOND EXPOSURE

TABLE 3.—*Root-decay analysis of 2-year-old trees*[a]

Series	Nutrient	Initial pH of nutrient	Roots of current season				Old roots			
			Roots	Dead	Infected	Lesions	Roots	Dead	Infected	Lesions
			No.	*Per cent*	*Per cent*	*No.*	*No.*	*Per cent*	*Per cent*	*No.*
A inoculated	Complete	4.50	265	1.4	2.4	0.0	354	4.8	4.2	3.0
B "	"	6.00	200	5.5	5.5	4.5	275	11.0	9.8	4.5
C "	"	7.00	374	12.6	13.4	6.5	531	31.8	11.0	5.0
D "	Low nitrogen	4.50	163	3.0	6.0	1.0	334	16.0	8.6	1.5
E "	Low phosphorus	4.50	161	2.0	5.2	0.0	492	22.0	5.3	6.0

[a] Based on eight inoculated plants in each series selected at random.

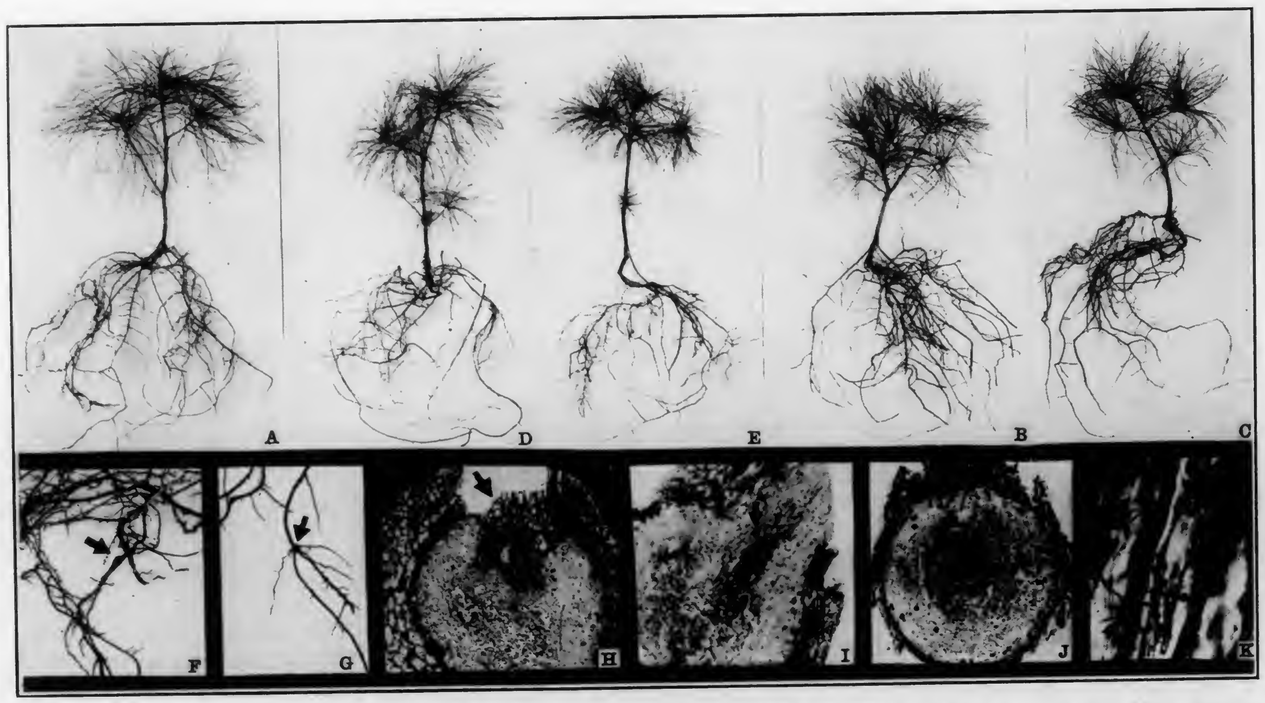

FIG. 2. Two-year-old trees from inoculated cultures. × ⅓. Series A, D, and E grown at pH 4.5: A, receiving the full nutrient; D, low nitrogen; and E, low phosphorus. Series B and C with plants receiving the full nutrient at pH 6.0 and 7.0, respectively. Note the similarity of growth response in A and B, and the long, sparsely branched root system of D. The small root systems of E and C are the result of heavy infection by the organism. F. Enlarged view of lesions on the roots of the plant in Series C. G. Excessive branching of parasitized roots. H. Typical entrance of the fungus at the base of a parasitized lateral root. × 40. I. Red discoloration in a lateral root extending toward the center of the mother root. × 25. J. Transverse section of a root, the stained area in the center composed of heavily lignified cells. × 25. K. The intracellular passage of *P. schweinitzii* within the tracheids of the parasitized root. × 500.

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			Roots	Dead	Infected	Lesions	Roots	Dead	Infected	Lesions
			No.	*Per cent*	*Per cent*	*No.*	*No.*	*Per cent*	*Per cent*	*No.*
A inoculated	Complete	4.50	265	1.4	2.4	0.0	354	4.8	4.2	3.0
B "	"	6.00	200	5.5	5.5	4.5	275	11.0	9.8	4.5
C "	"	7.00	374	12.6	13.4	6.5	531	31.8	11.0	5.0
D "	Low nitrogen	4.50	163	3.0	6.0	1.0	334	16.0	8.6	1.5
E "	Low phosphorus	4.50	161	2.0	5.2	0.0	492	22.0	5.3	6.0

[a] Based on eight inoculated plants in each series selected at random.

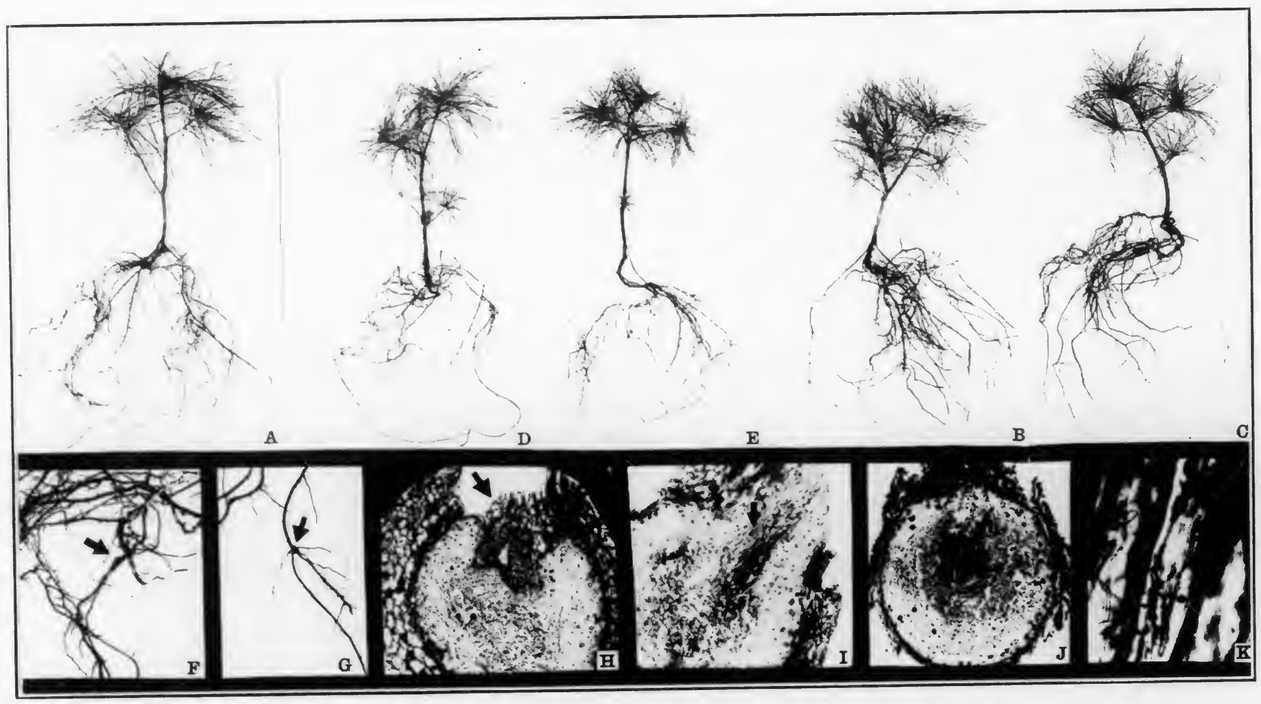

FIG. 2. Two-year-old trees from inoculated cultures. × ⅓. Series A, D, and E grown at pH 4.5: A, receiving the full nutrient; D, low nitrogen; and E, low phosphorus. Series B and C with plants receiving the full nutrient at pH 6.0 and 7.0, respectively. Note the similarity of growth response in A and B, and the long, sparsely branched root system of D. The small root systems of E and C are the result of heavy infection by the organism. F. Enlarged view of lesions on the roots of the plant in Series C. G. Excessive branching of parasitized roots. H. Typical entrance of the fungus at the base of a parasitized lateral root. × 40. I. Red discoloration in a lateral root extending toward the center of the mother root. × 25. J. Transverse section of a root, the stained area in the center composed of heavily lignified cells. × 25. K. The intracellular passage of *P. schweinitzii* within the tracheids of the parasitized root. × 500.

acterized by short laterals of larger diameter than those of the other series. The percentage of infection on the roots of the current season was similar to that of the low nitrogen series, but that of the older roots was similar to that of Series C at pH 7.0 (Fig. 2, E).

It is significant that all inoculated cultures in the various series yielded plants with less new-shoot growth and smaller root systems than were found under similar conditions in the controls. Fewer lateral roots, likewise, developed in the former cultures, with the exception of the 2-year-old plants of Series A.

The results from pH determinations of juice extracted from the roots of both age classes of the plants showed a correlation with the shift in pH of the nutrient solution. Juices from the needles and stems, however, failed to show any substantial deviations.

Infected roots (Fig. 2, K) showed injury of various degrees of severity. In some instances the mother roots bore lesions (Fig. 2, F) with large open areas of decay. In others it appeared that certain root tissues had become meristematic in an attempt to wall out the fungus. Direct infection had often taken place through epidermal and cortical cells (Fig. 3, A and B), although the typical mode of entrance appeared to be either through a lateral or at its base (Fig. 2, H and I). Portions of roots killed by the fungus showed a dark red discoloration of the central region (Fig. 2, J) extending upward in the mother root toward the root-crown, occasionally even into the stems. The fungus could not be isolated from tissues very far distant from the point of initial infection, even though the discoloration in the central portion of the root extended beyond such areas.

Chemical Analyses.[5] York (*op. cit.*) found upon comparing the ash analyses of wood from normal white pine with that of trees infected with *Polyporus schweinitzii*, that the diseased trees contained less calcium than the healthy ones. The results from ash analyses[6] of plants in the inoculated and control cultures are shown in table 4.

The outstanding points in these results are the correlation in the absorption of calcium and phosphorus and the greatly reduced amount of calcium in plants grown at pH 7.0. This would appear to confirm the belief of Truog (32), namely, that the feeding power for phosphorus is related to the calcium content of the plant.

The fact that York (*op. cit.*) found, as a result of several hundred pH tests and analyses of water extracts of the soil, that there was on the average one-third more calcium in areas of diseased trees than in disease-free plantings suggested the possibility that *Polyporus schweinitzii* may inhibit the entrance of calcium into the roots of trees. Chemical analyses were made

[5] These data were loaned to the writer through the courtesy of H. H. York.

[6] The chemical analyses were made by H. J. Hallowell, Consulting Chemist, Philadelphia, Pa.

TABLE 4.—*Ash analyses of seedlings and sand, indicated on a percentage basis of carbon and CO_2 free ash*

Series	Seedlings							
	Total ash		SiO_2		CaO		P_2O_5	
	1[b]	2	1	2	1	2	1	2
A[a]	3.89	3.99	2.48	7.90	8.72	12.93	23.75	21.49
A–c ...	4.02	3.50	2.29	7.89	10.08	11.05	23.76	28.75
B	3.94	3.03	2.39	7.07	10.02	12.02	23.51	21.93
B–c ...	3.74	3.31	3.14	6.82	10.63	16.80	25.87	27.05
C	4.20	3.33	3.77	8.68	6.23	8.35	18.35	21.99
C–c ...	5.32	2.81	4.20	6.23	6.72	13.88	21.35	24.75
D	4.06	2.93	1.60	5.25	9.86	7.68	25.38	22.02
D–c ...	4.48	3.22	1.51	6.80	10.34	10.84	28.63	25.82
E	4.14	2.87	2.66	8.68	7.93	7.59	22.85	20.02
E–c ...	4.61	3.32	2.22	9.40	10.80	15.40	26.76	25.28

Series	Sand
B—Inoculated	0.054% CaO
B—Control	0.0025% CaO

[a] A, B, C, D, and E—Inoculated; A–c, etc.,—controls, uninoculated.
[b] 1—Seedlings, 220 days old; 2—Two-year-old trees.

of water extracts for calcium in the quartz sand removed from the immediate vicinity of the roots of plants grown at pH 6.0. The results (Table 4) showed a percentage of only 0.0025 CaO in the control cultures, compared with 0.054 per cent about the roots of inoculated plants. Ordinarily, a root parasite is not considered as influencing the availability of nutrient material in the medium surrounding the roots of the host, but, under the conditions established in the sand cultures, it appears that *P. schweinitzii* caused the calcium to become less available to the plants.

Histological Study. Healthy and diseased roots were tested for cellulose and lignin content. For cellulose, potassium iodide with iodine and sulphuric acid were used, and for lignin, phloroglucin and hydrochloric acid. The infected roots showed a marked reduction in cellulose in the discolored areas, while the same zone evidenced heavy lignification of the cell walls. Compared with normal roots of similar age and size, the lignification of the woody elements was perceptibly advanced in the infected roots. It would thus appear that *Polyporus schweinitzii* produces an effect on the host that may hasten lignification of the cell walls (Fig. 3, C and D). Since the above tests gave similar results on tissue in which the hyphae were not present, but were discolored as a result of near-by infection, it seems that some product of the parasite was responsible for such host reaction.

When cross sections of diseased roots were stained with pH indicators, as that of the Hellige set and bromthymol blue of the Lamotte Chemical Company, the cell walls of the dead tissue acquired the color of the dyes in

their acid range, while the walls of the living, uninjured cells acquired the color of the dyes in their basic range. The acid color reaction also was found to occur upon treating normal white-pine wood known to be heavily lignified. These results indicate that the presence of *Polyporus schweinitzii* within host tissue causes a substantial change in the chemical constitution of the cell walls.

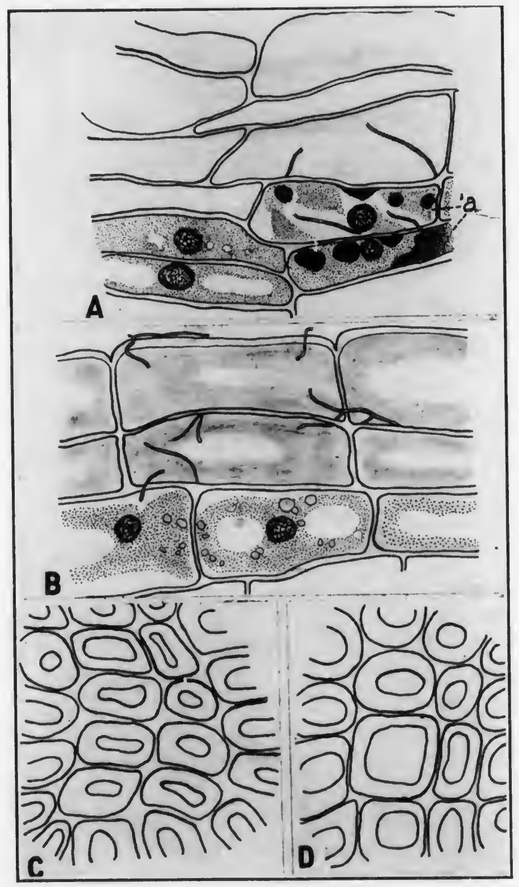

FIG. 3. Penetration of *Polyporus schweinitzii* into root tissue. Drawings made with the aid of a camera lucida. ×650. A. Hyphae in the living cells of a young root. Note the break-down in cell contents of cells "a" in contrast with adjoining cones unaffected by the presence of the fungus. B. The direct penetration of hyphae through the heavy cortical layer of cells in a 2-year-old root. C. Heavily lignified cells from affected tissue illustrating the thick walls and small lumen of xylem cells. D. Structure of conducting tissue in the root of a control plant.

An examination of stem tissue near the root-crown, into which a fungus filtrate had been injected by means of a capillary tube, showed death of the cells about the point of entrance and reddish streaks in the surrounding wood cells. This was in contrast with the normal meristematic action of tissue about wounds made by the injection of water and needle punctures.

Physiology of the Fungus. A number of media proved satisfactory for the pure culture of this fungus. Growth typical of *Polyporus schweinitzii* occurred on oatmeal agar, Trommer's plain malt agar, whole buckwheat, white-pine-needle duff, and noncrushed white-pine seed. Little growth occurred on rice, and none on potassium silicate plus nutrients that had been found satisfactory for growth when added to agar medium. Trommer's plain-malt agar proved to be the most satisfactory for root-isolations and the storage of cultures.

Liquid cultures of 2 per cent Trommer's plain malt showed growth over the wide pH range of 2.59 to 7.0. After a 14-day period, however, the solutions made above pH 4.0 were found to be decidedly more acid than when inoculated (Table 5). The fungus assumed a green-yellow at points lower than pH 3.5 and a brown-yellow as the conditions were more alkaline. The effect of the fungus upon the solutions in surface and submerged cultures was not comparable in the more alkaline pH range.

TABLE 5.—*Liquid culture data of Polyporus schweinitzii Fries.*

Item compared	Initial pH	pH after 14 days	Diameter of surface growth in mm.	Character of growth	Culture acid equivalent after 14 days when buffered. CC. N/10 acid
Surface cultures	2.59	2.55	50	sb.[a]	
	3.20	3.20	70	sr.	
	4.00	3.60	82	sr.	
	4.65	4.00	90	sr.	
	5.18	3.65	85	sr.	
	6.00	3.62	65	sr.	
	7.00	4.20	40	sr.	
Submerged cultures	2.59	2.55	.....	sb.	
	3.20	3.21	.....	sb.	
	4.00	3.60	5	sr.	
	4.65	3.55	45	sr.	
	5.18	3.49	7	sr.	
	6.00	4.32	.....	sb.	
	7.00	5.00	.....	sb.	
Buffered solutions vol. 37.5 cc.	2.0	remained the same	45	sb.	5.25
	3.0		50	sb.	6.00
	4.0		60	sb.	6.00
	5.0		2	sb.	3.75
	6.0		1	sb.	1.50
	7.0		.....	.....	.....
	8.0		.....	.....	.....

[a] Sb.—submerged growth, Sr.—Surface.

Liquid cultures, buffered by potassium hydrogen phosphate and citric acid, showed a sharply defined pH optimum for this fungus (Table 5). Growth occurred at pH 2.0, but none above 6.0, while 3.0 to 4.0 appeared to be the optimum. The titratable acidity of the solutions was determined before inoculation, and 14 days later, using 0.1N NaOH, with phenolphthalein as an indicator. The amount of acid was found to be increased in all solutions where growth had occurred. From chemical analyses, it appeared that the increase in acidity was due to the production of succinic acid by the fungus.

DISCUSSION

An attempt has been made in this study to correlate the parasitism of seedling white-pine roots by *Polyporus schweinitzii* with growth conditions in sand cultures. For this study the 2 drip methods of supplying the nutrient, irrrespective of the system, proved very satisfactory in maintaining growing conditions for the plants. The development of corky excrescences around points of emergence of laterals indicated that the sand had been kept well moistened (Fig. 1, F and G). Reactions to the modifications in the nutrient solutions were rather sharply defined, and it was quite evident that the growth response of young white pine can be determined best by a study of both shoot and root reaction.

There was a definite increase in the length of the root system and in the number of lateral roots as the nutrient solution was made more alkaline. However, with this increase in length and number of roots, the average root diameter became less, and thus the total root surface would not increase by so large a ratio as might seem to be the case. The development of the roots near the surface of the sand at pH 7.0 may have been due to the greater amount of phosphorus in that area. It is probable that a large percentage was precipitated into a less available form of ferric phosphate before penetrating far into the sand. Moreover, with the increase in alkalinity of the nutrient solution, the amount of calcium and phosphorus absorbed by the plants was decreased, and there was an increase in the alkalinity of the root extract. Since the susceptibility of white-pine roots to infection by this fungus appears in this experiment related to the chemical nature of the nutrient medium, the amount of calcium and phosphorus available must have an important bearing on host resistance. True (31) has contributed an explanation of the rôle played by calcium in the normal absorption of essential ions by plants, while Loew (17) has shown that phosphorus is of primary importance in the formation of new cells. At pH 7.0, the roots were typical of plants low in calcium (10), and reduced phosphorus proved to be a limiting factor to the increase in the size of seedling plants.

In the case of the older plants when grown at pH 4.5, a reduction in the amount of either phosphorus or nitrogen in the nutrient solution resulted in

greater extension of the root system. These results are in accordance with several previous studies (14, 19, 20, 24, 26). Low nitrogen in the case of the young seedlings produced a similar reaction, but low phosphorus unfavorably affected root length extension. The root systems of plants in the latter case were decidedly reduced in length, even though ash analyses showed phosphorus absorption to be nearly equal to that of the plants receiving the full nutrient at pH 4.5. It appears that the reduction in amount of available nitrogen and the increase in alkalinity of the nutrient solution affect the extension of the root systems of young white pines in similar manner.

The length of the shoots of the young seedlings was about equal, regardless of the acidity, when receiving the full nutrient. The older plants were more sensitive, however, with the least new shoot growth of the 5 series occurring at pH 7.0. Low nitrogen did not impair shoot development of the younger plants, although the stem diameter was less. A reduction of phosphorus suppressed shoot growth of young seedlings, as did nitrogen and phosphorus in the case of the older plants. From the above it appears that the effect of nutritional variations influences the development of roots more than that of the shoots of 1-year-old white-pine seedlings, while older plants may be expected to show rather sharply through their shoot development the influence of the medium in which they are grown.

The needles, regardless of the age of the plants, showed similar color reactions to the modifications in the nutrient solution. The needles of plants receiving the full nutrient at pH 4.5 were apparently normal in color. The reduction of phosphorus resulted in a deeper shade of green, but not a purple-green, as has been reported for phosphorus-starved plants. The yellow-green formed at pH 6.0 and 7.0 seemed to indicate low availability of iron.

The results of this investigation show that *Polyporus schweinitzii* is capable of parasitizing the roots of seedling white-pine trees under the conditions of this study. Parasitism occurred on both roots of the current and previous seasons' growth. Injury was evidenced by death of the small roots, the production of lesions, and red discoloration in larger roots. The presence of the fungus decidedly reduced the total length and number of lateral roots on the root systems of inoculated plants. Entrance into living host tissue was found to occur directly through epidermal and cortical cells, through the base of lateral roots, and through the corky excrescences at the base of some of the lateral roots. The intracellular passage of this fungus from one host cell to another occurred without evidence of mechanical pressure or constriction of the hyphae. Its entrance into the living roots of seedling plants indicates the possibility that this fungus may be distributed by nursery stock to forest plantings, and, furthermore, that it may be a factor affecting the survival of naturally reproduced white pine and possibly other conifers.

The seedlings grown under alkaline conditions and also those with low phosphorus and nitrogen were the most susceptible to attack. Hence, it appears that where these conditions exist singly or together, *Polyporus schweinitzii* may be expected to be especially destructive. The effect of this fungus upon absorption of mineral elements by its host is important, since the ash of infected plants showed a lower percentage of calcium than that of the controls, and an analysis of water extract of the sand from inoculated cultures showed a higher percentage of calcium than in the controls. Whether the calcium was made less available by the presence of the fungus, or whether the plants for some reason were unable to absorb the element, is problematical; the former interpretation seems the more logical, however, since it appears that the fungus produces succinic acid in liquid cultures.

Since an acid was produced by *Polyporus schweinitzii* during growth on buffered solutions, the red discoloration that occurred within infected roots may have been due to the acid liberated by the parasite within the host tissue. Furthermore, it seems that this excretion caused prematurity and death of the cells it penetrated. This may explain the observations on structural weakness of wood beyond the areas of visible decay (5). That this fungus produces a substance causing wood discoloration and change in cell character is further indicated by the fact that reddish streaks developed when a filtrate prepared from the mycelium was injected into woody tissue of 3-year-old white-pines.

SUMMARY

A physiological and pathological study of white-pine seedlings, inoculated with *Polyporus schweinitzii,* has been made in order to determine whether the fungus may become parasitic to living root tissue.

Root and shoot growth of the plants was found to be correlated with the pH of the nutrient solution and reductions of inorganic nitrogen and phosphorus. Low phosphorus proved to be a limiting factor in young seedling growth, while the reduction of nitrate nitrogen did not unfavorably affect it.

The absorption of calcium and phosphorus was reduced at pH 7.0 in both control and inoculated cultures. The presence of the fungus lowered the absorption of calcium by inoculated plants, as evidenced by the occurrence of relatively large amounts of calcium compounds in the immediate vicinity of the roots and a lower calcium content in the plant ash.

The hyphae of this fungus were found penetrating directly through living cortical cells of the root and corky excrescences at the base of lateral roots.

Parasitism increased with the alkalinity of the nutrient solution and with the reduction of phosphorus.

Infection of roots was accompanied by a reddening of host tissue. Such areas seemed to be prematurely lignified in young roots, and appeared to be acid in character. The total length of the root system and the number of lateral roots were less in the case of infected plants.

The parasitism of living roots on plants of seedling age indicates the possibility of this fungus being spread by nursery stock to forest plantings and, further, indicates that it is a factor affecting the natural reproduction of susceptible host species.

Polyporus schweinitzii, in liquid cultures, produces an acid capable of increasing the acidity of nonbuffered solutions. On buffered solutions, the optimum point for growth is pH 4.0.

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A Hybrid-fern Name and Some New Combinations

EDGAR T. WHERRY[1]

In the course of compilation of a popular guide to the ferns of the Middle Atlantic States, which it is hoped may be published in the near future, there arose the need for certain nomenclatorial novelties which are here placed on record.

Asplenium ebenoides R. R. Scott is now generally accepted to be a hybrid between *Asplenium platyneuron* and *Camptosorus rhizophyllus*. Article 32 of the International Rules of Botanical Nomenclature, 1930, states that bigeneric hybrids are to be designated "whenever it seems useful or necessary, by a name," consisting of "a new 'generic' name usually formed by a combination of the names of the parent genera." In accordance with this plan, it is now proposed to designate the fern in question as ×**Asplenosorus ebenoides** (Scott), nom. hybr. nov. As an appropriate common name, Walking Spleenwort may be used.

[1] Contribution from the Botanical Laboratory and Morris Arboretum of the University of Pennsylvania.

Botrychium obliquum var. *pennsylvanicum* Graves. Does not differ essentially from the original *B. obliquum* Muhl. ex Willd., which was, indeed, described from Pennsylvania; will be taken up as representing the earliest name for this in varietal status. Two other well-marked varieties are var. *oneidense* (Gilbert) Waters and var. *tenuifolium* (Underw.) Gilbert. There are also many forms, one of which requires further discussion. This is a mutant with the marginal serrations more or less intensified, and, as shown by Tryon,[2] may develop from the same root which another year produces a normal *obliquum* frond. It has been regarded as the plant to which Sprengel gave the name *B. dissectum,* and, when reduction to the status of form is made, the rules of priority require the combination to read *B. dissectum* f. *obliquum,* as pointed out by Clute. The more southern plant to which Sprengel's name was applied is, however, much more deeply cut, with divergent teeth, and grows independently; in the writer's opinion it is to be classed as a distinct species. Anyone who feels that forms must have technical names can coin a new one for the serrate *obliquum.*

When a species has become segregated into two or more geographic varieties—or subspecies, as they are coming to be termed by an increasing number of authors—all of the segregates should be regarded as of equal rank and assigned trinominals. This has already been done for some of our eastern species, but apparently not for those named below. When there is no name in varietal status already on record, the Rules permit, for the subdivision which includes the type, either a duplication of the species name or the use of "one of the customary epithets, *typicus, genuinus, originarius,* etc.," the latter alternative being preferred here.

[2] This Journal **26**: 26. 1936.

Botrychium simplex var. **cordatum** (Fries), comb. nov. *B. lunaria* var. *cordatum* Fries. This is the variety represented by the original *B. simplex* Hitchcock. Others are var. *tenebrosum* (A. A. Eaton) Clausen and var. *laxifolium* Clausen.

Osmunda claytoniana var. **vera,** nom. nov. The basis of *O. claytoniana* L. A varietal name is needed to make this coordinate with the Asiatic var. *vestita* (Wall.) Milde.

Adiantum pedatum var. **originarium,** nom. nov. The plant named *A. pedatum* L. It grades into var. *aleuticum* Rupr.

Pteridium latiusculum var. **verum,** nom. nov. The original *P. latiusculum* (Desv.) Hieron. ex Fries. Another is *pseudocaudatum* (Clute) Maxon.

Onoclea sensibilis var. **genuina,** nom. nov. The American *O. sensibilis* L., with an Asiatic counterpart, var. *interrupta* Maxim.

Athyrium angustum var. *typicum* (Butters), stat. nov.[3] *A. a.* forma *typicum* Butters. The epithet is needed in varietal status for coordination with vars. *elatius, rubellum,* etc.

Lycopodium lucidulum var. **verum,** nom. nov. The variety on which was based *L. lucidulum* Michx. Its counterpart is the more northern var. *occidentale* (Clute) Wilson.

Lycopodium inundatum var. **typicum,** nom. nov. The basis of *L. inundatum* L. Here there are two other varieties, var. *bigelovii* Tuckerm. and var. *robustum* R. J. Eaton.

Equisetum sylvaticum **var.** *multiramosum* (Fernald) stat. nov. *E. s.* var. *pauciramosum* forma *multiramosum*

[3] The category term is placed in bold face here because it is the novelty, rather than the third epithet; the latter is sometimes so treated even when merely changed in status, but in my opinion this may be misleading.

IRREGULAR PAGINATION

Fernald. Since I interpret the term form as applying to a peculiar plant which appears sporadically in the midst of normal ones, and variety to a plant of abundant occurrence over a definite range, I can only place this Horsetail in the latter category.

PHILADELPHIA, PA.

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January–March, 1938, pages 28–30.

Midland Fern Notes

Edgar T. Wherry

During the summer of 1937 the writer drove from Philadelphia to Salt Lake City and back, visiting en route a number of fern localities; Mr. J. E. Benedict, Jr., of Washington, D. C., took part in the first half of this trip. Notes as to species observed which seem of general interest are here placed on record, except for the state of Colorado, on which a separate article has been prepared. Specimens obtained have been placed in the herbarium of the Academy of Natural Sciences of Philadelphia.

Michigan.—We barely entered this state late in May, paying a visit to Mr. and Mrs. Boydston, who live at Thorn Acres about four miles south of Niles, in Berrien County. Here in ponds and on sandy slopes at the edge of the floodplain of the St. Joseph river there is an unusual abundance of Equisetums, of which five species were recognized: *arvense, fluviatile, laevigatum, palustre,* and *prealtum.* This appears to represent a southern limiting station for *E. palustre* (var. *americanum*), for although it is here within a mile of the Indiana line the thorough exploration of the latter state by Mr. Deam has never turned it up there.

Iowa.—A visit to Pikes Peak State Park in Clayton County yielded *Selaginella rupestris,* not heretofore reported from this county, and two varieties of *Cystopteris fragilis.* Examination of their rootstocks and indusia showed these to be vars. *genuina* and *protrusa,* the former growing on steep bluffs, the latter in humus-rich soil on gentler slopes.

At Decorah search was made for the two rarities there reported. One, *Phegopteris robertiana,* was soon found west of town on a moist, crumbly, north-facing limestone bluff; unfortunately it is in danger of destruction, since the road close to which it grows leads to a state park, and may at any time be "improved" by landscrapers. The other, *Woodsia scopulina,* could not be located, and in view of the dominance of limestone in the region its occurrence there seems improbable.

The *Woodsia* which grows on the quartzite rocks in Gitchie Manitou State Park, in Lyon county at the extreme northwest corner of the state, has been reported both as *W. scopulina* and *W. oregana*; but a visit there disclosed that it is neither, as it shows instead the dense glandularity characteristic of *W. cathcartiana.*

South Dakota.—There being reports of both *Woodsia oregana* and *W. mexicana* in the Black Hills, some attention was paid to this group there, especially in the Custer State Park. No trace of the glabrous fern bearing the former name, or of the downwardly narrowed, thick-margined, conspicuously hairy-indusiate Mexican Cliff-fern could be found. Instead the plants seen were all more or less intermediate between these two extremes, the margins being more or less thickened—never markedly so—and short-stalked glands being present on vascular parts, but sparse or lacking on laminar tissue. Here, as in western Iowa, *W. cathcartiana* seems unquestionably represented, although some material appears to lie intermediate between that and some other species, and will require detailed study for proper classification and naming.

Oklahoma.—During August a brief visit was made to Cimarron, the northwesternmost county, to look for the

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unusual ferns which have been reported there.[1] From Kenton highway No. 64 was followed eastward for 6 miles, and a private lane northward about a mile farther, until cliffs became prominent. The native vegetation in low moist places proved to have been largely destroyed by grazing animals, making search for *Dryopteris filix-mas* hopeless, but small ferns could still be seen in crevices of the sandstone rocks. North-facing cliffs and ledges yielded *Cheilanthes eatoni, C. feei,* and *C. wootoni,* together with *Selaginella underwoodii,* not previously recorded for this region. On south-facing ones the yellow globules formed by the rolling up of the fronds of *Notholaena standleyi* were conspicuous, and while examining these several small but unmistakable plants of *Pellaea wrightiana* were found.

TEXAS.—In the hope that some of these Cimarron valley specialties might also turn up in the Texas panhandle, the Palo Duro State Park east of Canyon, Randall County, was next visited. Here, however, only the widespread *Cheilanthes feei* and *Pellaea atropurpurea* could be found on the rocks, and *Equisetum prealtum* along the creek.

LOUISIANA.—Under the guidance of Miss Caroline Dormon, of Chestnut, three species not listed by Brown[2] for this state were obtained: *Cheilanthes lanosa,* in crevices of sandstone north of Kisatchie (15 miles south of Provencal); *Selaginella riddellii,* in a sand-barren 2 miles southeast of Goldonna, both in Natchitoches Parish; and *Lycopodium prostratum,* in a wet thicket 1½ miles north of Lucky, Bienville Parish.

PHILADELPHIA, PA.

[1] Greene, THIS JOURNAL 17: 125. 1927; Bush. Am. Midl. Nat. 12: 91. 1930; Featherly and Still, Okla. A. & M. Coll. Expt. Sta. Circ. 80. 1934.

[2] La. Cons. Rev. 5: 12. 1936.

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Vol. 19, No. 2, pp. 433-435, March, 1938

A New Phlox from the Snake River Canyon Idaho-Oregon

Edgar T. Wherry and Lincoln Constance

In the course of the study of the genus *Phlox* being carried on by the senior author, the existence of a hitherto undescribed member of the genus in the Snake River region was recognized. Specimens were found to be preserved in several herbaria, variously identified as *P. linearifolia, P. longifolia,* or *P. whitedii,* although it differs markedly from these. Its nearest relative is *P. speciosa* var. *nitida* Suksdorf, with which it agrees in glabrous herbage and short styles, but on the average its leaves are narrower, flowers fewer, pedicels longer, calyx-membranes more carinate, and corolla-lobes longer. Moreover, while in all normal *P. speciosa* the corolla-lobes are notched, becoming entire only in rare mutants, in the present plant these lobes are consistently notchless and terminally obtusish, acutish or mucronate. In addition, it occupies a range remote from that of the Pacific-border var. *nitida,* so may well be classed as an independent species. A name for it has been derived from one of the Latin terms for snake, in reference to its region of occurrence.

Phlox colubrina sp. nov.

Plant a slender glabrous shrub 15 to 50, averaging 30 cm. tall; leaves thinnish or with somewhat thickened margins, narrowly linear; the largest 40 to 80, average 50 mm. long, and 1 to 2 (rarely 2.5), average 1.5 mm. wide; inflorescence 1 to 6 or rarely 12-flowered; pedicels 18 to 70, average 35 mm. long; sepals 8 to 12, average 10 mm. long, united ½ to ⅔ their length; membranes of calyx-tube rather distinctly carinate, the lobes glabrous externally but pilose on their inner surface; corolla-tube 9 to 15, averaging 11 mm. long, sometimes constricted near its orifice, externally glabrous; lobes 9 to 20 by 4 to 7 mm., usually 2 to 3 times as long as wide, terminally varying from obtusish to acutish or to mucronate; stamens deep within the tube, rarely less than 3 mm. below the orifice; styles 1.5 to 4, averaging 2.5 mm. long, free and stigmatic for 1/3 to 2/3 their length.

Frutex parvus, glaber; folia circa 1.5 mm. lata; corollae lobi latitudine 2-3-plo longiores obtusiusculi, acutiusculi vel mucronati; styli 1.5-4 mm. longi.

Type in Herbarium Academy of Natural Sciences of Philadelphia, collected by Lincoln Constance, H. Heggeness, D. Hedrick, and D. Peters, No. 1823, May 13, 1937, 1 mile above the mouth of Sheep Creek, a tributary of the Snake River, Idaho County, Idaho, latitude 45° 27½′ N., longitude 116° 32½′ W.

Other collections known to date are listed herewith, the herbaria being abbreviated to groups of letters which obviously represent them:

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IDAHO. Idaho Co.: Snake River Canyon 2 m. n. of Sheep Cr., 45°29′, 116°33½′, Constance, Rollins & Dillon 1595, 5/16/'36 (Wash. St. and ANSP); mouth of Willow Creek, 45°29½′, 116°33½′, Constance, Hedrick & Peters 1821, 5/13/'37.

OREGON. Wallowa Co.: near mouth of Battle Creek, 45°18½′, 116°40½′, Peck 18143, 3/27/'34, (Will. U. & ANSP); 4 m. ne. of Buckhorn Spr., 45°48′, 116°47′, Peck 18286, 6/27/'34 (Will. U. & ANSP); headw. left fk. Cache Cr., 45°59′, 116°58′, Sheldon 8185, 5/28/'97 (NYBG, USNH); Cache Cr. Bar, alt. 800′, 45°59′, 116° 54½′, Constance, Rollins & Dillon 1548, 5/14/'36 (Wash. St. & ANSP); Cherry Cr. at 2000′, 45°50′, 116°50′, Jardine 388, 4/5/'09 (U. S. For. Serv.); Snake Canyon near Deep Cr., 1000′, 45°47′, 116°40′, C., R., & D. 1567, 5/15/'36 (Wash. St. & ANSP); Imnaha, 45°33½′, 116°50′, Sherwood 73, 6/4/'23 (Will. U. & Field M.); m. Imnaha R., 45°49′, 116°46′, G. N. Jones 6440, 4/11/'31 (U. Wash. & ANSP); n. slope Mt. Wilson, 45°49′, 117°5′, Sheldon 8094, 5/20/'97 (NYBG, USNH).

Fig. 1. *Phlox colubrina* in the Snake River Canyon.

Fig. 2. The type specimen of *Phlox colubrina*.

This is the common *Phlox* of the Snake River Canyon and its tributary streams from about the mouth of the Grand Ronde River to the north edge of Hell's Canyon, where the river cuts its channel between the Wallowa and Seven Devils ranges. Doubtless the species extends still farther south but the canyon has not been explored above this point. Within this deep trough, *Phlox colubrina* ranges through a variety of habitats, appearing to be equally at home in the timbered and timberless Arid Transition and the badly overgrazed Upper Sonoran Zone. A list of its associates would comprise a considerable proportion of the highly endemic canyon flora. In the timber belt it is found under *Pinus ponderosa* and *Amelanchier Cusickii* in company with

Castilleja angustifolia, Brodiaea Douglasii and *Arabis crucisetosa,* and spreads down through the bunchgrass climax with these and other herbs. Perhaps its frequency is highest on the dry basaltic ledges of the Upper Sonoran whose sparse vegetation is dominated by *Celtis Douglasii, Amelanchier Cusickii, Rhus glabra, R. Toxicodendron, Ribes Gooddingii* and *Opuntia polyacantha.* In the most severely grazed sectors, the *Phlox* often persists solely within the protective presence of *Opuntia* (see Fig. 1) and *Rhus glabra,* surrounded by such unpalatable herbs as *Amsinckia, Leptotaenia, Cogswellia fragrans, Erigeron concinnoides* and *Lupinus sericeus.* Plants of the last habitat, growing in crannies in the volcanic rock, are usually more obviously shrubby and more numerous flowered than the lax and sprawling, few-flowered individuals found in the partial shade of shrubs. The soil reaction is practically neutral (pH 6.8 to 7.2).

Corolla color is somewhat variable, but most of the fresh material showed the outside of the lobes and tube to be (terminology according to Ridgway) "Pale Laelia Pink," and the inside to range from "Light Rosolane Purple" to "Rosolane Purple." The lobes are frequently bimaculate at the throat with deeper hues of the same color, and a few plants were found with pure white corollas. The flowers of *Phlox longifolia* Nutt., a species which replaces *P. colubrina* in the Snake River Canyon in the vicinity of the mouth of the Clearwater River, have the lobes "Pale Rose-Purple" outside and "Light Mallow Purple" within.

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AND
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BERKELEY, CALIFORNIA.

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INTENTIONAL SECOND EXPOSURE

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Vol. XI, 1937, pages 52–54.

NOTABLE PENNSYLVANIA FERNS

By Edgar T. Wherry
University of Pennsylvania

Although the early botanists did not usually designate a type locality for their species, there seems reason to believe that at least ten of our native ferns may be regarded as having been first described from this State. These are:

Asplenium ebenoides R. R. Scott. Described in 1865 from the southwest bank of the Schuylkill River, in Montgomery County.

A. pinnatifidum (Muhl.) Nuttall. This was discovered by Muhlenberg, no doubt in southern Lancaster County, and named by him *A. rhizophyllum* B. *pinnatifidum* in 1813. It was raised to species rank by Nuttall 5 years later.

A. trudelli Wherry. Founded in 1925 on specimens collected at Cully, just below Holtwood, along the Susquehanna River in Lancaster County.

Botrychium obliquum Muhl. ex Willd. Muhlenberg collected this, in all probability in Lancaster County, and sent it to Willdenow, who described it in 1810.

Dryopteris intermedia (Muhl. ex Willd.) Gray. This had the same history as the next-preceding, although it was at first mistakenly described as a *Polypodium,* transfer to the genus *Dryopteris* being made by Gray in 1848.

Lygodium palmatum (Bernh.) Swartz. Another discovery by Muhlenberg; some reference to it is made in his journal, now preserved at the American Philosophical Society in Philadelphia, but just where he obtained it is not clearly stated. He sent it to Willdenow, who announced it as a *Hydroglossum,* but this name was first published as a synonym for *Gisopteris palmata* by Bernhardi in 1801. Five years later Swartz transferred it to his genus *Lygodium.*

Lycopodium obscurum L. John Bartram collected this near Philadelphia and sent it to Europe, where it was figured by Dillenius in 1741. Linnaeus based his species upon this figure. Another Clubmoss with the same early history was misunderstood by Linnaeus, and grouped under

the northern *L. complanatum,* not being recognized as deserving species standing until 1919, when Blanchard named it *L. flabelliforme.*

Polystichum acrostichoides (Michx.) Schott. Michaux observed this fern in "Pennsylvania, Carolina, and Tennessee" and named it *Nephrodium acrostichoides* in 1803. Schott transferred it to *Polystichum* 31 years later.

Selaginella apoda (L.) Fernald. This was probably sent to Europe by Bartram, for when he figured it in 1741, Dillenius gave the range as "Pennsylvania, Virginia, and Carolina." When Linnaeus named it *Lycopodium apodum* in 1753, he listed the same States, but in reverse order. Fernald made the correct combination in 1915.

Woodsia obtusa (Spreng.) Torrey. When Sprengel named this *Polypodium obtusum* in 1804, he attributed it merely to Pennsylvania, but as he is known to have been receiving material from Muhlenberg at the time, the probabilities are that it had been collected by the latter, in Lancaster County. Transfer to the correct genus was made by Torrey in 1840.

Several ferns are noteworthy in that, although in part discovered elsewhere, they reach a range-limit in this State. Here may be enumerated:

1. Circumboreal Plants

Botrychium multifidum (var. *silaifolium*). This enters from the north, being known in Erie and Monroe Counties, and reaching a limit in central Berks County. (One station in Monmouth County, New Jersey, being slightly farther south.)

Botrychium simplex. Known in 10 counties: Berks, Bucks, Crawford, Dauphin, Indiana, Lehigh, Monroe, Northampton, Pike, and Wayne. A station near Finland, Bucks County—lat. 40° 23′ N.—is apparently the southernmost known for the species. Three varieties are represented, but their individual distributions have not been worked out.

Cryptogramma stelleri. In cool ravines from western Lycoming to eastern Wayne counties. Known further south only at one place in Bergen County, N. J.

Polystichum braunii (var. *purshii*). Found in ravines tributary to that of Kitchen Creek in western Luzerne County, and to that of Fishing Creek in eastern Sullivan County; also near Lake Shehawken, Wayne County. The first named is the southern limit for the species, lat. 41° 18′ N.

Equisetum litorale. The taxonomic status of this plant is not clear, but at any rate it reaches its southern limit here, along the Susquehanna River in York and Lancaster Counties, and at Chester in Delaware County.

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2. Appalachian Mountain Plants

Lygodium palmatum. The Climbing Fern has a sporadic distribution, but becomes locally abundant in several northeastern mountain counties, reaching a western limit near Lopez, Sullivan County, long. 76° 20′ W.

Asplenium bradleyi. Several stations along the Susquehanna valley in York and Lancaster Counties and one in Carbon County. The latter is a northwestern limiting occurrence, although the species is known at two places each in New Jersey and New York. *A. gravesii,* the hybrid between this and the next, reaches its northeastern limit in Lancaster County.

Asplenium pinnatifidum. Scattered through the more southern counties, Lancaster County being in the lead, with 15 recorded stations. An old specimen from Berks County, if authentic, represents the northeastern limit of the species, reports from New Jersey apparently referring to the next following.

Asplenium trudelli. One station in Fayette, and several each in York and Lancaster counties. This presumable hybrid between *A. pinnatifidum* and *A. montanum* reaches a northern limit in Warren County, N. J.

3. Southern Calcareous Rock Plants

Asplenium resiliens. This Mexican species follows limestone outcrops into Missouri and North Carolina, and has been especially successful in traversing the Shenandoah valley. It recently turned up on a single cliff near Mercersburg, in Franklin County, Penna., lat. 39° 47′ N., apparently the absolute limit of its range.

4. Eastern Bog Plants

Thelypteris simulata. The northwesternmost authentic occurrence of the Bog Fern is that in Bear Meadows, Centre County, long. 77° 45′ W. Reports farther northwest have proved to be based on misidentifications. Little is known as to the limits of the range of this plant in the south, and it may have entered the continent from a former land mass lying off our coast, now buried beneath the sea.

Lycopodium adpressum. Besides being found along the Delaware, this coastal lowland species extends inland to White Oak, in northern Lancaster County.

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NOTABLE PENNSYLVANIA ORCHIDS

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Last year there was presented a discussion of the ferns native to Pennsylvania which are of special interest in having been discovered in this State. A similar study of the native orchids is now offered.

Aplectrum hyemale (Willdenow) Nuttall. This species was collected by Muhlenberg, no doubt in Lancaster County, and sent to Willdenow under the manuscript name *Cymbidium hyemale*, which was published by the latter in 1805. Nuttall based a new secondary genus *Aplectrum* on it in 1818, this being raised to full genus standing by Torrey in 1826.

Corallorrhiza wisteriana Conrad. Early taxonomists rarely gave definite localities for their plants, but the present species was exceptional in that its describer stated it to have been collected by Charles J. Wister in 1828 along the Schuylkill River "between the Falls and the mouth of the Wissahickon Creek," in what is now northwestern Philadelphia County.

Cypripedium candidum Muhlenberg ex Willdenow. Muhlenberg's manuscript name for this was published by Willdenow in 1805, the source being merely stated as Pennsylvania. It may well have come from a locality in Lancaster County designated on a label accompanying a specimen collected by A. P. Garber in May, 1865 as "Little Conestoga near Millersville." (Porter Herbarium, now at the Academy of Natural Sciences of Philadelphia.) There is no other record of the species in the State.

Habenaria blephariglottis (Willdenow) Hooker. This orchid was early found by Clayton in Virginia, but never described on his material. In announcing it under the name *Orchis blephariglottis*, Willdenow ascribed it to Pennsylvania, without recording its exact locality or collector. Since sphagnum swamps, in which it grows, are widespread, it may have come from almost any part of the State. Assignment to the genus *Habenaria* was made by Hooker in 1824.

Habenaria orbiculata (Pursh) Torrey. When he described this species under the name *Orchis orbiculata* in 1814, Pursh knew it "on the mountains of Pennsylvania and Virginia." Torrey transferred it to the genus *Habenaria* in 1826.

Habenaria viridis var. *bracteata* (Willdenow) Gray. Being unfamiliar with European orchids, Muhlenberg considered this plant, which he presumably found in Lancaster County, an independent species, and named it *Orchis bracteata.* The same view was held by Willdenow, who published this name in 1805; by R. Brown, who transferred it to the genus *Habenaria* in 1813; and by Parlatore, who placed it in the supposedly distinct genus *Coeloglossum* in 1858. Meanwhile, in 1851, Reichenbach recognized it to be a mere variety of the circumboreal species which he termed *Platanthera viridis.* His view is now coming to be accepted, although following Gray (1867) we now use the genus name *Habenaria* for it.

Isotria verticillata (Willdenow) Rafinesque. (*Pogonia v.* of Gray's Manual). This species was listed as from Maryland by Plukenet in his Mantissa, published about 1700, and figured in his plate 348, but received no name for over 100 years. Then Muhlenberg collected it, no doubt in Lancaster County, and named it *Arethusa verticillata;* this name was published by Willdenow in 1805, his diagnosis being based chiefly on the Pennsylvania material. Three years later Rafinesque made it the type of his genus *Isotria,* and he is now followed by most botanists, although some prefer the assignment to *Pogonia,* made by Nuttall in 1818.

Orchis obsoleta and *O. virescens* Muhlenberg ex Willdenow. These supposed species were collected by Muhlenberg in Lancaster or neighboring counties, and sent to Willdenow who published their names in 1805. They apparently represent variations of the comprehensive species named by Linnaeus from Virginia *Orchis flava,* and now known as *Habenaria flava.* The first Muhlenberg name is usually ignored, but Fernald has published the second in varietal status under *H. flava,* in 1921.

Certain species are of interest in another way, namely in reaching significant range-limits in Pennsylvania; they may be listed under three geographic headings:

1. NORTHERN SPECIES

Goodyera tesselata (*Epipactis t.* of Gray's Manual, ed. 7.) When Loddiges described this species in 1824, he stated it to have been received from "New York and Philadelphia," but these were merely shipping points. Four Pennsylvania stations for it are known, the southernmost in Huntingdon County, latitude 40° 40′.

Habenaria dilatata. A specimen in the Porter herbarium, now at the Academy of Natural Sciences of Philadelphia, from Union

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City, Erie County, latitude 41° 55′, represents a southern limit for this species.

Habenaria hookeri. This is known from a number of northeastern and central stations, the southernmost being at Bedford Springs, Bedford County, latitude 39° 50′. It probably also ranges into West Virginia.

Habenaria hyperborea. Porter had specimens of this from three counties, the southernmost station represented being Mt. Pleasant, latitude 41° 44′, in Wayne County.

Malaxis brachypoda (formerly known as *Microstylis monophyllos.*) This occupant of cold calcareous swamps enters the State from the northeast, and extends down along the higher mountains. Its limit here is represented by a sheet in the Porter herbarium labelled "Summit of the Alleghenies, Blair County," which would place it at latitude 40° 30′.

Spiranthes romanzoffiana. The southernmost record for this orchid is one in the Porter herbarium from Harmonsburg, Crawford County, latitude 41° 40′.

2. Southern Species

Corallorrhiza wisteriana. In addition to having been discovered in Pennsylvania, this coral-root is of interest in reaching its northern limit here, near Oley Furnace, Berks County, latitude 40° 20′.

Habenaria cristata. Although most abundant on the Coastal Plain, the Lesser Orange Fringe-orchid locally invades more northern or upland provinces. Its northwesternmost station was in Fraser's Bog, Montgomery County, lat. 40° 5′, long. 75° 5′.

Isotria affinis (*Pogonia a.* of Gray's Manual.) Because of its erratic behavior, in appearing at any one station only at intervals of several years, the range of this species is but incompletely known; however in view of its greater abundance and more consistent reappearance in southeastern North Carolina and Virginia, it is regarded as a southern plant. Its westernmost record is that in Greene County, Pennsylvania (*Trillia* 7:44, 1923). Interestingly enough, Muhlenberg's herbarium contains a specimen of it, labelled as a variety of his "*Arethusa verticillata.*"

Listera reniformis (*L. smallii*). A southern Appalachian Twayblade, reaching its northern limit in Bear Meadows, southeastern Centre County, latitude 40° 45′.

Malaxis bayardi. This rare relative of *M. unifolia,* distinguished by its very short pedicels and correspondingly narrow raceme,

has been known heretofore only from two stations in the mountains of North Carolina and from Nansemond County, near the coast of Virginia, from where it was described by Fernald in 1936 (*Rhodora* 38:402). In the University of Pennsylvania herbarium there are specimens of it from Schuylkill and Luzerne counties, identification of which has kindly been confirmed by Professor Fernald. Thorough examination of herbarium sheets here and elsewhere has failed to show any collections farther north.

Spiranthes beckii. Though chiefly a Coastal Plain plant, this tiny Spiral-orchid occasionally enters the uplands, and has been collected in Pennsylvania as far northwest as Gibraltar, Berks County, lat. 40° 17′, long. 75° 53′.

3. Midland Species

Cypripedium candidum. The White Slipper-orchid is classed as a Midland plant, in that its principal range is from Kentucky and Missouri north to the Great Lakes. Locally, however, it extends eastward across New York to northern New Jersey. The Lancaster County station where the species was discovered, already referred to above, represents a southeastern limiting occurrence, lat. 40° 0′, long. 76° 22′.

THE PHLOXES OF OREGON

BY EDGAR T. WHERRY.

For some years the writer has been collecting data on the genus *Phlox*, but so complex are the problems involved that there seems no hope of completing a monographic treatment in the near future. In order that certain taxonomic findings thus far made can be included in the Flora of Oregon which is under preparation by Professor Morton E. Peck, of Willamette University, the following notes on species and subspecies (designated by simple trinomials) known to occur in that state are being published. The plants are taken up in three groups, respectively those with tall erect stems and short styles; with like stems and long styles; and with short branched stems yielding cespitose clumps.

Erect-stemmed, Short-styled Group

Phlox speciosa Pursh.

The variant of this northern species on which the name was based, *P. speciosa euspeciosa* Brand, is characterized by tall habit (average height 35 cm.), glabrate lower herbage, and thinnish long-acuminate leaves. It grows chiefly in Idaho and Washington, barely entering Oregon in the neighborhood of The Dalles. From eastern Washington southwestward it grades into a derivative with lower stature (average height 25 cm.) and slightly thickened, short-acuminate leaves, which has never received subspecies classification, so is here termed *P. speciosa* **occidentalis** (Durand) Wherry.[1] The absence of this from northern and central Oregon is apparently due to its having been destroyed there by Tertiary volcanic activity, for it is frequent in the southwest corner of this state, and southward into California.

Two variants of *Phlox speciosa* with thick, glandular leaves have developed in central Washington, and extend a short distance into Oregon. One may be known as *P. speciosa* **lanceolata** (E. Nelson) Wherry;[2] it averages 28 cm. tall, with long internodes, and few lanceolate leaves up to 30 to 60 mm. in length. The other was termed by E. Nelson *P. whitedii*, but Brand's name *P. speciosa lignosa* is the earliest in subspecies status. It differs in averaging but 20 cm. tall, the internodes being short and the leaves

[1] Status novus: *P. divaricata* var. *occidentalis* Durand, J. Acad. Nat. Sci. Phila. [2] 3: 97. 1855; *P. occidentalis* Durand ex Torrey, Rept. Expl. & Survey RR. 4: 125. 1857; *P. speciosa* . . . f. *occidentalis* Brand, in Engler's Pflanzenreich IV. 250: 74. 1907.

[2] Status novus: *P. lanceolata* E. Nelson, Rev. W. N. Am. Phlox: 29. 1899; *P. speciosa* . . . f. *lanceolata* Brand, in Engler's Pflanzenreich IV. 250: 73. 1907.

IRREGULAR PAGINATION

numerous, linear to lanceolate, and up to 15 or exceptionally 30 mm. long. Material from Tygh Valley, Oregon, is gradational between these, but typical specimens of the second were distributed from the state's "eastern prairies" by Howell.

Another variation from the ancestral *P. speciosa* stock consists in loss of pubescence from the entire plant except the inner surfaces of sepals, resulting in *P. speciosa* **nitida** (Suksdorf) Wherry,[3] in southern Washington. This migrated southward into California, but as was the case with its immediate ancestor, *P. s. occidentalis*, it vanished from northern and central Oregon, and survives in this state only toward the southwest corner.

Phlox colubrina Wherry and Constance.

While similar to *P. speciosa nitida* in the limitation of pubescence to inner sepal-surfaces, this *Phlox* has leaves averaging only 1.5 mm. wide (as against 4 mm. in most subspecies of *P. speciosa*); moreover its corolla-lobes are unique in the group in being elongate and acutish to mucronate, those of *P. speciosa* being normally short, obtuse, and notched. It has accordingly been described recently as a new species.[4] It is endemic in Idaho and Oregon along the Snake River canyon, in the latter state also extending well up into the northeastern mountains.

Erect-stemmed, Long-styled Group

Phlox adsurgens Torrey ex Gray.

The type locality of this species was Canyon Pass, south of Canyonville, Douglas County, Oregon. A visit there in 1931 showed it to be abundant as a ground-cover in thin woodland, the diagnostic character given for it by Brand, "stolons lacking," being erroneous. It is closely related to the eastern *P. stolonifera* Sims, which also has well-developed creeping stems with broad leaves, the two having no doubt arisen from a common ancestor which grew in the far north in pre-Glacial times. Northern occurrences were destroyed, however, by the ice, and the limiting station at present known is 8 miles south of McKenzie Bridge, Lane County, Oregon.

Phlox viscida E. Nelson.

Diagnostic features of this *Phlox* comprise the presence of glandular pubescence nearly throughout the herbage, and flat intercostal membranes of the calyx. Brand associated it with *P. stansburyi*, but it differs markedly from that southern species. It is endemic in the mountains of southeastern Washington and adjacent Oregon.

[3] Status novus: *P. speciosa* var. *nitida* Suksdorf, Deutsch. botan. Monatsb. 18: 32. 1900; *P. speciosa* . . . subvar. *nitida* Brand, in Engler's Pflanzenreich IV. 250: 74. 1907.

[4] Amer. Midl. Nat. 19: 433. 1938.

Phlox longifolia Nuttall.

This widespread northern *Phlox* is characterized by the presence of conspicuous bulges in the intercostal membranes of the calyx, often termed replications, but more aptly described as carinae. Several of its variants have been classed as independent species, and the fact that they sometimes occur in pure stands lends some support to this procedure. In numerous colonies, however, two or more occur so intermingled that they can only be regarded as extremes in a variable series, so subspecific status is here assigned to five of them.

A variant ranging from moderately dwarf to tall and slender, with the upper herbage densely glandular-pubescent and the largest leaves around 50 to 100 mm. long is regarded as the ancestral one. Recombining the earliest name assigned to it leads to its being termed *P. longifolia* **longipes** (Jones) Wherry.[5] From this arose a usually dwarfer and consistently short-leaved extreme—maximum fertile-stem leaf-length 25 to 45 mm.,—which may be known as *P. longifolia* **compacta** (Brand) Wherry;[6] this has been supposed to have the calyx membranes only obscurely carinate, but the type specimen does not bear this out. At the type locality this grows intimately admixed with two or three other subspecies, all blooming simultaneously.

Another direction in which variation occurs is in the nature of the pubescence; this may remain abundant, but become wholly eglandular, without change in the plant's stature or leaf-length. Such was the variant on which the original species description was based; it was also represented in Hooker's *P. speciosa* var. *linearifolia*, and Brand named it *P. longifolia linearifolia*, which is here adopted. As in the glandular material, another variant has on the average lower stature and shorter leaves; this was first collected by Douglas, and may be known as *P. longifolia* **humilis** (Douglas ex Hooker) Wherry.[7] Finally, the pubescence may tend to thin out, ultimately yielding plants which are wholly glabrous except within the sepals. St. John[8] has recently regarded this as the original *P. longifolia*, overlooking Nuttall's use of the term puberuli in characterizing that species. The glabrous subspecies has never been assigned a name, so may be known as:

[5] Status et comb. nova: *P. linearifolia* var. *longipes* Jones, Contr. W. Botany 12: 53. 1908; *P. longifolia* var. *filifolia* A. Nelson, Bot. Gaz. 54: 143. 1912.

[6] Comb. nova: *P. virida* E. Nelson, Rev. W. N. Am. Phlox: 24. 1899; *P. longifolia* var. *puberula* ibid. 26; *P. stansburyi compacta* Brand, in Engler's Pflanzenreich IV. 250: 67. 1907; *P. puberula* A. Nelson, in Coulter's Man. Bot. C. Rocky Mts.: 397. 1909.

[7] Status novus: *P. humilis* Douglas ex Hooker, Flora Bor.-Amer. 2: 72. 1838; *P. speciosa* β Hooker, loc. cit.; *P. longifolia* . . . var. *humilis* Brand, in Engler's Pflanzenreich IV. 250: 66. 1907; *P. cernua* E. Nelson, Rev. W. N. Am. Phlox: 22. 1899.

[8] Torreya 36: 94. 1936.

Phlox longifolia calva Wherry, subsp. nova.

Differing from all other variants of the species in having the herbage wholly glabrous, except inside the sepals. Plant 10 to 50 cm. tall; largest leaves 45 to 90 mm. long and 2 to 4 mm. wide. Text-fig. 1.

Fig. 1. Phlox longifolia calva, subsp. nova. Habitat view of the plant selected as the type.

Planta tota glabra, sepalis interne exceptis, 10 ad 50 cm. alta; folia maxima 45 ad 90 mm. longa et 2 ad 4 mm. lata.

Type collected by Edgar T. Wherry June 21, 1931, 13 miles southwest of Darlington, Custer County, Idaho, in herbarium Academy Natural Sciences Philadelphia. Range, central Washington to western Montana, south to Utah and Colorado. May occur mingled with one or more of the other representatives of the species, but locally forms pure stands.

All five subspecies here recognized occur scattered over northeastern Oregon, as far west as Mono, Shaniko, and Tygh Valley. Many colonies include two or more of them, with intermediates between extremes.

Cespitose Group

Phlox douglasii Hooker.

Phototype and clastotype material of this northern Phlox in American herbaria shows it to be an open-cespitose plant averaging 15 cm. high, with the herbage abundantly beset with long septate gland-tipped hairs. Unfamiliar with its features, E. Nelson applied to an unusually large but otherwise typical specimen of it the name *P. piperi*. It occurs chiefly in the Columbia Plateau region of eastern Washington and Oregon, with an alpine extreme in the Cascades. Reports from other regions are based on what are here regarded as wholly distinct species.

The subspecies on which the name *douglasii* was based has been named by Brand ssp. *eudouglasii;* its type locality was the Blue Mountains. In bleak or dry places this grades into a plant differing chiefly in being lower in stature—average height 5 cm.—which has stiffish leaves and was named by Bentham *P. rigida*. Gray referred this to a variety of the Rocky Mountain *P. caespitosa*, but the glandularity of its leaves leads to its reclassification here as *P. douglasii* **rigida** (Bentham) Wherry.[9] This occurs in several places in the Columbia Plateau.

Above tree-line in the Cascades further evolution occurs; the leaves become more appressed and somewhat shorter (average maximum length 7.5 mm.) and the styles have an average length of but 2.5 mm. This is the plant termed by E. Nelson *P. condensata* var. *hendersoni*. Its resemblance to *P. condensata* may well be due merely to their occupying similar high-alpine situations, and in pubescence it is more like the *P. douglasii* series. Did it not intergrade with ssp. *rigida* it might be assigned species status, but it is more safely classed as *P. douglasii* **hendersoni** (E. Nelson) Wherry.[10]

From the Phloxes just discussed *P. caespitosa* Nuttall differs in having the glandularity limited to the inflorescence. Its various subspecies occur largely in the Rocky Mountains, but it may possibly enter eastern Oregon locally.

Phlox covillei E. Nelson.

This species differs from the others in the Great Basin region in having tiny thickish leaves of elliptic-lanceolate outline, only 3 to 5 times as long as wide. Its herbage is pubescent, but only a portion of the hairs are gland-tipped. It has been known heretofore from California and Nevada, but Professor Peck has collected what appears to be a variant of it above Ice Lake in the Wallowa Mountains of Oregon.

Phlox lanata Piper.

The type locality of this northern *Phlox* was Steins Mountain, Harney County, Oregon; it is now known to extend also into Montana. It is pulvinate in habit, with long prostrate leafy shoots. The herbage, except toward the tips of leaves and sepals, is covered by copious tomentose hairs, and even the corolla-limb may bear minute pubescence.

[9] Status novus: *P. rigida* Bentham, in DC. Prodromus 9: 306. 1845; *P. caespitosa* var. *rigida* Gray, Proc. Amer. Acad. Arts Sci. 8: 254. 1870.

[10] Status et comb. nova: *P. condensata* var. *hendersoni* E. Nelson Rev. W. N. Am. Phlox: 14. 1899; *P. caespitosa* . . . var. *hendersonii* Brand, in Engler's Pflanzenreich IV. 250: 84. 1907.

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INTENTIONAL SECOND EXPOSURE

Phlox diffusa Bentham.

In describing this northern *Phlox* from near the southern end of its range in California in 1849, Bentham suggested it to be related to *P. douglasii*, but the characters he gave to distinguish them were not well chosen, no reference to their difference in pubescence being made. Gray proceeded to reduce *diffusa* to varietal status, and has been followed by many subsequent workers, leading to great confusion. Phototypes and clastotypes of *P. diffusa* show it to be a spreading plant, pubescent with long wholly eglandular hairs; its leaves are thinnish, linear but not sharp-pointed, and

Fig. 2. Phlox diffusa longistylis, subsp. nova. Habitat view of the colony from which the type specimen was selected.

reach a length of 15 mm. In the upstanding *P. douglasii*, on the other hand, the hairs are gland-tipped, and the leaves are thickish, subulate with a sharp point, and rarely as much as 10 mm. long. If any division of the cespitose Phloxes (which Gray considered "almost inextricable") is to be made at all, species differentiation has to be based on characters such as these. *P. douglasii* and *P. diffusa* are accordingly here maintained as independent.

Phlox diffusa is common from the hills of western British Columbia southward along the Cascades to the Sierras of California, where it is limited

to fairly high altitudes. Toward the northern end of this range its styles are 6 to 12 mm., toward the south 3 to 5 (rarely 7) mm. long, the boundary between the two variants lying in northern Oregon. The southern one was the original *P. diffusa*, and may accordingly be named *P. diffusa* **typica** Wherry.[11] Nearly all specimens seen from western Oregon belong here. The northern extreme deserves a subspecies name, as follows:

P. diffusa longistylis Wherry, subsp. nova.

Differing from ssp. *typica* in somewhat lower stature and smaller leaves and flowers, and especially in having longer styles, 6 to 12 mm. in length. Text-fig. 2.

A subspecies typica differt planta paulo minor et stylis 6 ad 12 mm. longis.

Type collected by the writer July 30, 1931, at 7250 feet altitude on the south slope of Mt. Adams, Yakima County, Washington, in herbarium Academy Natural Sciences Philadelphia. Range, British Columbia south to House Mountain, Marion County, Oregon.[12]

Phlox hoodii Richardson.

While this species was described from the plains of Saskatchewan, and is especially frequent in the high plains and eastern foothills of the Rockies, it also crosses these mountains in Idaho and spreads over the Great Basin region. The phase most developed in the latter area differs from the original in having a slightly longer corolla-tube, and was named *P. canescens* by Torrey and Gray. The differences between them are so slight and the intergradation so complete, however, that only subspecific independence seems justified, leading to the new combination: *P. hoodii* **canescens** (T. & G.) Wherry.[13] This subspecies occurs sporadically in the Columbia Plateau region of Oregon, extending up to the eastern foothills of the Cascades. It thus approaches the range of *P. diffusa*, from which it differs in its more compact habit, and smaller leaves and flowers.

Phlox austromontana Coville.

While originally described from southern Utah, this *Phlox* with its distinctive acerose leaves and carinate calyx-membranes is wide-ranging over the Great Basin and adjoining physiographic provinces. In Oregon it has been collected as far north as Union County. A long-styled phase of it

[11] Nomen novum: *P. diffusa* Bentham, Plant. Hartweg.: 325. 1849; *P. douglasii* var. *diffusa* Gray, Proc. Amer. Acad. Arts Sci. 8: 254. 1870.

[12] When I first encountered this *Phlox* and observed its differences from the original *P. diffusa*, I supposed it might prove to be *P. caespitosa* Nuttall, and so reported to St. John and Warren, who have included it under that name in "The Plants of Mt. Rainier National Park, Washington". Amer. Midl. Nat. 18: 978. 1937. Actually the Mt. Rainier *Phlox* is this newly described *P. diffusa longistylis*.

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INTENTIONAL SECOND EXPOSURE

was described by Brand as *P. densa,* but this is scarcely separable; his *P. pinifolia,* so far as can be ascertained, is another minor variant of the same species.

Phlox peckii Wherry, sp. nov.

Plant a prostrate under-shrub; main root branching above into several creeping woody stems, sending up erect branches 1 to 3 cm. tall, yielding pulvinate-cespitose clumps; internodes short, their surface laminated, more or less pubescent; leaves spreading, thinnish, linear, 4 to 8 mm. long and 0.5 to 1.5 mm. wide (ratio of length to width 5 to 10), basally densely ciliate with septate hairs, superficially granulate to glabrous; the tip bearing a short cusp; inflorescence 1-flowered, the pedicel 1 mm. long, quadrate; sepals 8 mm. long, united about ½ their length, the costa prominent, awn-tipped; intercostal membranes distinctly carinate; pubescent with long kinky septate eglandular hairs, on the outside near the base and on the inside of the lobes toward the tip; corolla-tube about 12 mm. long, somewhat dilated upward; lobes cuneate, 5 mm. long and 2.5 mm. wide, terminally erose-mucronulate, their upper surface beset to a distance of 3 mm. from the orifice with fine kinky septate hairs up to 0.5 mm. long; upper part of tube yellowish, limb cream-color; stamens extending rather high up the tube, one or two anthers slightly exserted; styles 4.5 mm. long, united to ⅘ their length, the 3 stigmas thus 1 mm. long; ovules solitary. Text-fig. 3.

Fig. 3. Phlox peckii, species nova. Flower × 2, showing unique pubescence.

Fruticulus prostratus; folia linearia, ad 8 mm. longa et 1.5 mm. lata; calycis membranae carinatae, tubo baso eglanduloso-pubescente; corollae lobi superne pubescentes, pilis ad 0.5 mm. longis; styli 4.5 mm. longi, ad ⅘ longitudinis conjuncti.

Type collected by Professor Morton E. Peck of Willamette University, Salem, Oregon, in whose honor the plant is named: No. 19302, July 14, 1936, dry slope, north rim of Crater Lake, Oregon. Type in Herbarium Academy Natural Sciences Philadelphia.

This *Phlox* is obviously a derivative of *P. diffusa,* in which the flat to wrinkly calyx-membrane of the parent species has become rather definitely carinate (as it has in *P. austromontana* also). In most Phloxes the corolla limb is glabrous, but in a few of the cespitose ones it is minutely granulate, and in *P. lanata* occasionally fine-puberulent. The present species, however, is the only one known in which the corolla-limb is definitely pubescent.

Of the 12 species discussed in the above pages, 6 are northern in geographic relationship, being best developed in the northern Rocky Mountains, and enter Oregon from the northeast; 3 are southern, ranging over the Great Basin, and occupying southeastern Oregon; while the remaining 3 are endemic in Oregon, or in this state and adjacent portions of surrounding ones.

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Southern Occurrences of Dryopteris Clintoniana

EDGAR T. WHERRY[1]

On June 1, 1927, Mr. Harry W. Trudell and the writer, out on a camping trip, stopped to shave at a limpid, purling brook in the woods 1½ miles east of Forney, Cherokee County, Alabama. Most unexpectedly we found that just back of the spot we had selected there was growing a *Dryopteris* of the general aspect of *D. clintoniana,* though differing from the more northern material of this fern in certain respects. The photograph of it taken at the time is here reproduced in Plate one; plants growing near-by in more open situations showed more erect fronds.

Subsequent herbarium study showed that a similar plant had already been collected in the state: it was discovered about 1895 by the late Professor S. M. Tracy, in a needle-palm swamp said to be 4 miles south of Auburn in Lee County, and was referred by Mohr[2] to *D. floridana;* from this it differs, however, in numerous respects, notably in having the frond widest well below the middle, without any conspicuous, sudden change in degree of cutting where sterile pinnae give way to fertile ones, and in having the lower pinnae acuminate with a sharp tip

[1] Contribution from the Botanical Laboratory and Morris Arboretum of the University of Pennsylvania.

[2] Plant Life of Alabama: 317. 1901.

and the marginal teeth sharp-pointed. I have been unable to relocate this colony, but collections made there by Pollard and Maxon in 1900 are widely distributed in herbaria. More recently, ferns of more or less the same features have been found in Arkansas, Louisiana, and North Carolina.

At intervals during the subsequent ten years I have repeatedly tried to find some feature which would separate this fern sharply from *Dryopteris clintoniana,* but have never succeeded. Only varietal status seems possible for it, as follows:

Dryopteris clintoniana (Eaton) Dowell, var. **australis,** var. nov. A varietate boreali differt soris plusminusve medialibus. Differing from the northern plant in having the sori more or less medial, instead of rather near the midvein. Sometimes, though by no means consistently, tending to develop fertile pinnae only above the middle of the blade, and sinuses in the upper ones nearly as wide as the adjacent segments; the northern variety often showing fertile pinnae near the blade-base, and sinuses at most $\frac{1}{3}$ as wide as segments.

Type in herbarium Academy Natural Sciences Philadelphia, collected by Edgar T. Wherry July 15, 1932, in humus-rich subacid woodland soil $1\frac{1}{2}$ miles east of Forney, Cherokee Co., Alabama.

The northern plant, which now requires a varietal name, may become:

Dryopteris clintoniana (Eaton) Dowell, var. **genuina,** nomen novum.

Records of the southern variety known to date are:

ALABAMA.—The plants of both colonies above referred to show marked variation in rhizome scales, from pale brown and dull to nearly as dark brown and lustrous as in *D. goldiana;* the specimen selected as type exhibits the latter extreme, but a cotype sheet, bringing out the rootstock features (with attached sterile fronds), has

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DRYOPTERIS CLINTONIANA VAR. AUSTRALIS AT FORNEY, ALA.

pale scales. Pinnae may lie close together, as in the frond shown at the right of Plate 1, or again may be spaced as widely as is normal in var. *genuina*. The sinuses between fertile segments are usually very narrow, as in the frond pictured, and also the type specimen, but on occasional vigorous plants may be wider.

ARKANSAS.—Specimens from rocky woods just north of Shirley, Van Buren Co., have been distributed by the discoverer, E. J. Palmer, and also by Demaree and Moore. Some of these have much the aspect of the Alabama material, and the sori lie well out, being at least medial or even slightly supra-medial. Here too the scales vary from the pale brown of *D. clintoniana* to the dark and shining ones of *D. goldiana*. The latter is approached also in one other respect—the basal pinnae may have their lowermost segments shortened, so that their outline is somewhat lanceolate. Sinuses between lobes vary from narrow to wide, the fronds in extreme cases becoming bipinnate.

LOUISIANA.—Early in 1936 Dr. Clair A. Brown discovered a colony 6 miles southeast of Baton Rouge, which I visited in September. Here the plants approach var. *genuina* in having sori beginning to appear not far above the base of the blade, and occupying a slightly inframedial position. The sinuses between fertile lobes show, however, a marked tendency to widen, so the occurrence is classed as var. *australis*.

NORTH CAROLINA.—Material collected by Rev. Fred W. Gray 9 miles north of Charlotte, Mecklenburg Co., has been figured by Blomquist.[3] This shows an approach to *D. floridana* in elliptic frond-outline, the lower 4 pairs of pinnae being progressively shortened, but is *clintoniana* in every other respect. In the narrow sinuses

[3] Ferns of N. C.: 65. 1934.

DRYOPTERIS CLINTONIANA VAR. AUSTRALIS AT FORNEY, ALA.

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[3] Ferns of N. C.: 65. 1934.

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between fertile lobes it is like var. *genuina,* but the medial sori shown in the figure cited place it in var. *australis.* Variation occurs in the latter feature, however, for in fronds recently sent by Mr. Gray to the Philadelphia Academy the sori are distinctly infra-medial.[4]

Two possible explanations of the remarkable variability shown by the plants under discussion suggest themselves. One is that they represent the result of hybridization between several species. The occasional dark scales and lanceolate basal pinnae would indicate *D. goldiana* to have been one of these. Tendencies toward basally narrowed blade, development of fertile pinnae only terminally, and widening of sinuses between fertile segments all point to *D. floridana* having been another. Movement of the sori out from the midvein suggests the entry into the complex of *D. marginalis.* Finally, the outlines and cutting of the pinnae correspond most often to *D. clintoniana genuina.*

The alternative explanation, which is favored here, is that we are dealing with a stock which has already given rise to several descendants, and is still undergoing evolution. *D. clintoniana genuina* and *D. goldiana* represent, then, offshoots which migrated northeastward, while *D. floridana* is one which moved coastward. In these, features which appear in the ancestral stock as mere uncorrelated tendencies became so definitely combined and stabilized that we can recognize one distinct variety and two independent species.

PHILADELPHIA, PA.

[4] Although full discussion of more northern variants is beyond the scope of the present article, record should be made of the fact that material of *D. clintoniana* from as far north as Dauphin and Berks counties, Pennsylvania, shows an approach to var. *australis* in sorus-position. On the other hand, var. *genuina* is known down to Dade Co., Georgia (Pyron and McVaugh, 1936).

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THE JOURNAL
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Southern Appalachian Botanical Club

Vol. 2. January, 1937 No. 1.

Two Plant-geographic Notes[1]

EDGAR T. WHERRY

Thuja occidentalis in Pennsylvania and Maryland. — In a series of maps of ranges of trees published by Transeau,[2] dots are placed in counties of Ohio and adjacent states where the species are supposed to grow. That these maps are incomplete as far as eastern Pennsylvania is concerned has already been noted.[3] The map for *Thuja* is also unsatisfactory for the Appalachian region. On map No. 17, covering this plant, but one Pennsylvania county, Crawford, has received a dot. In the herbarium of the Carnegie Museum at Pittsburgh, however, specimens are preserved from two other counties in this state, namely Armstrong and Westmoreland. No dots at all are allotted to Maryland, but in 1934 I collected *Thuja* on the limestone cliffs along the old canal 2¾ miles southwest of Sharpsburg, Washington County;[4] a specimen has been placed in the herbarium of the Morris Arboretum. Thus, instead of there being a marked discontinuity between the northern range of this tree and its occurrences in the southern Appalachians, the two regions are connected by a chain of colonies.

Pseudotaenidia montana in Pennsylvania. — The Mountain-pimpernel was described by Mackenzie[5] in 1903 from Kates Mountain, West Virginia, and about the same time was collected by Steele near Luray, Virginia,[6] but for many years was known only from these two stations. Recent exploration in northwestern Virginia by Professor R. S. Freer and others has shown it to be rather widespread there. It

[1] Contribution from the Botanical Laboratory and Morris Arboretum of the University of Pennsylvania.
[2] Ecology 16: 429-35, 1935.
[3] Bartonia 17: 51, 1936.
[4] Amer. Fern Journ. 24: 99, 1934.
[5] Torreya 3: 159, 1903.
[6] Gray's Manual, ed. 7: 620, 1908.

grows on various formations, including sandstone and limestone, although it becomes most abundant on the barrens underlain by hard Middle Devonian shale rock. On following these shales northeastward, it was found on hills along the North Branch of the Potomac River three miles south of Ridgley, Mineral County, northeasternmost West Virginia. Search in Maryland then disclosed a small colony on a shale hill one mile north of Oldtown, Allegany County; this has been noted in Claytonia.[7] It was subsequently found at several points along the crest of Polish Mountain, in the same county.

During August, 1936, an effort was made to find it still farther north. Some 7 miles southwest of Chaneysville, in Bedford County, Pennsylvania, there are fairly well developed shale barrens, and the plant was found here at two places. Both lie within sight of the telephone wire marking the Mason and Dixon line, but unquestionably within the state. One occurrence consisted of a single plant on road bank on west slope of small hill east of Iron Ore Ridge; the other, of half a dozen plants, beside a little used road which runs along the west flank of Ragged Mountain, and south of the point marked Elbinsville on the topographic map ascends toward the crest of Polish Mountain. Search at more northern points has thus far disclosed only *Taenidia integerrima,* a far more widespread plant, identical in herbage, but with the fruit having the two carpels subcylindric instead of lenticular as in *Pseudotaenidia.*

UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA.

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INOCULATIONS WITH FOREST TREE RUSTS

HARLAN H. YORK

In June, 1927, near Woodgate, N. Y., several species of pine and of the Scrophulariaceae were inoculated with the aeciospores of the *Peridermium* known as the Woodgate rust. The inoculations were kept moist by the use of a celluloid "iceless refrigerator," which is a modified form of the inoculation chamber described by Hubert.[1]

The 1927 terminal leaders, side shoots, and side branches of 5- to 20-year-old pines were moistened thoroughly and were smeared with aeciospores by means of a small artist's brush. A celluloid cone, which was 22 inches in length and respectively 3 inches and 1–1½ inches in diameter at the ends, was slipped over the inoculated parts. It was anchored to the stem by means of a copper wire. A strip of absorbent cotton, about 1 inch in width and 8–10 inches in length, which was saturated with water, was then pushed by means of a small rod through the smaller end, well down the inside of the cone. The entire outer surface of the cone was wrapped to a thickness of 1–1½ inches with wet absorbent cotton. The larger end of the cone was left almost entirely open, the smaller almost entirely closed, leaving an opening sufficiently large for circulation of air through the cone. If the cone was on the terminal shoot of the main axis of the tree, it was supported by one or two sticks, wired to the trunk of the tree; if on a side branch, it was supported from the ground by means of a forked stick (Fig. 1). The cones were left on the shoots for 24 to 36 hours. At the end of 36 hours the cotton on the outside of the cone was quite damp and it was always possible to wring water from the strip of cotton inside the cone. The entire outer surfaces of the shoots were also quite damp.

About 200 shoots of *Pinus resinosa, P. sylvestris, P. radiata,* and *P. ponderosa* were inoculated by this method. In all cases, infection spots appeared on the stems within four to six weeks, and in many cases, on *Pinus sylvestris,* the spots were so abundant that the entire inoculated stem surface became reddish brown in color by the latter part of August. This was equally true of trees which had no galls on them at the time the inoculations were made and of those which had galls.

At the time the shoots were inoculated, comparative germination tests of aeciospores from collections of various ages were made in the inoculation chambers and in water cultures in the laboratory. The water cultures were made by dusting the spores on fresh well-water in wine glasses. In the inoculation chambers pieces of thoroughly clean, white China silk about 2 cm. in length and 1 cm. in width were laid on the pine stems and the aecio-

[1] HUBERT, ERNEST E. Celluloid cylinders for inoculation chambers. Phytopath. 6: 447–450. 1916.

spores were dusted on them. The silks were examined under the microscope 24 to 36 hours later. The germ tubes were very much branched and anastomosed so much with one another that they formed very complicated mycelia. In the water cultures, the germ tubes of the same age were comparatively unbranched and unanastomosed. In all of these experiments the percentage of germination in the inoculation chambers was far greater than that in the water cultures. For example, the germination of spores which had been kept in Syracuse watch glasses in the laboratory away from direct sunlight for about 10 days was approximately 25 per cent as compared with 75 per cent in the inoculation chambers.

FIG. 1. Celluloid "iceless refrigerator" used in inoculation experiments with the aecio spores of the Woodgate rust on *Pinus sylvestris* and other species of hard pines.

It is possible that Woodgate rust may prove to be one of the western gall rusts, whose alternate hosts are species of the Scrophulariaceae. Although several species of Scrophulariaceae occur in the region where the Woodgate rust has been found, none of them has ever been found infected with any rust. During the latter part of June, 1927, *Chelone glabra* and *Scrophularia leporella* were inoculated with aeciospores of the Woodgate rust. These two species were selected because of their size and because they occur nearby.

The plants when inoculated were about 26 inches in height. Both sides of the leaves were thoroughly moistened and smeared with aeciospores as mentioned above for pines. A stake about ¾ inch in diameter, wrapped

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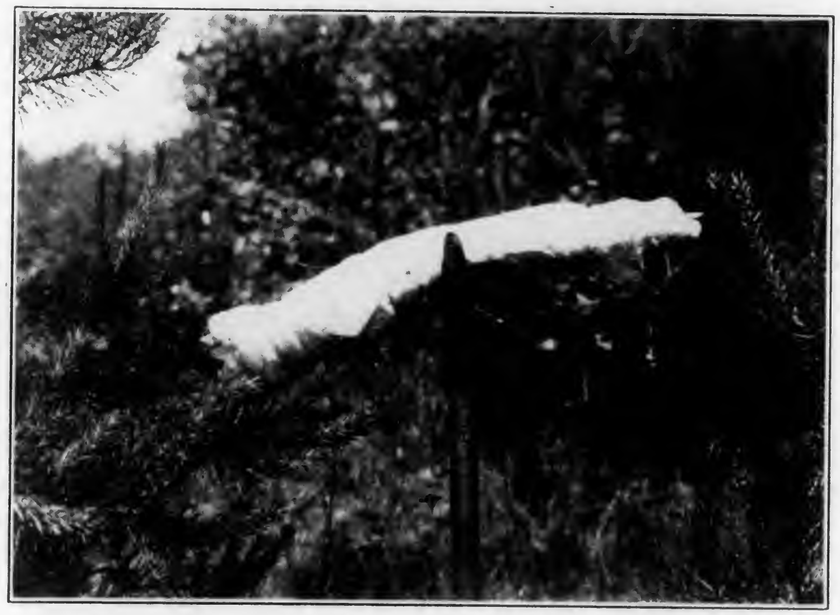

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with strips of wet absorbent cotton to a thickness of 1½ inches was thrust into the ground beside the plants, so that its upper end extended 4 inches above them. A celluloid cylinder, 22 inches long and 5 inches in diameter, was placed over the plants and stake, and rested on the ground. It was wrapped with wet absorbent cotton, which extended almost to the tip of the stake so as to leave the end of the cylinder partly open. After 48 hours, the cotton on the stake was wet and the leaves were quite moist. Microscopic examinations were made of pieces of the inoculated leaves soon after the cylinders were removed. The aeciospores had germinated as abundantly and the germ tubes were as profusely branched and had fused as freely as described above. No infection resulted in any of these experiments.

The writer and his assistants have made several thousand field inoculations with aciospores, urediniospores, and sporidia of *Cronartium ribicola,* using the celluloid cones, the celluloid cylinders previously mentioned, and the iceless refrigerators of the type described by Hunt[2] and Snell.[3] The results with the celluloid cylinders were almost entirely negative; those with the "Hunt iceless refrigerator" were fairly satisfactory, though less so than anticipated. Considerable labor is involved in using the "Hunt iceless refrigerator" in the field, especially in carrying water. The celluloid "iceless refrigerator" described above is the most convenient, dependable and labor-saving device which the writer has used thus far for field inoculations with forest tree rusts.

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[2] HUNT, N. R. The "iceless refrigerator" as an inoculation chamber. Phytopath. 9: 211–212. 1919.

[3] SNELL, W. H., and ANNIE RATHBUN-GRAVATT. Inoculation of *Pinus strobus* trees with sporidia of *Cronartium ribicola.* Phytopath. 15: 584–590. 1925.

Reprinted from SCIENCE, May 28, 1937, Vol. 85, No. 2213, page 528.

ACETO-CARMIN MOUNTING MEDIA

BELLING'S aceto-carmin technique,[1,2] which has been so valuable for a quick examination of meiotic divisions, has proven to be especially useful in the investigation of the giant chromosomes in the salivary glands of the Diptera. The chief disadvantage of the method lies in the fact that the preparations are temporary and the specimens can not be preserved without further treatment. Often specimens so prepared are too valuable to be discarded and a number of methods have been devised for transforming these temporary preparations into permanent slides. (McClintock,[3] Steere,[4] Buck,[5] Marshak,[6] etc.). These methods serve the purpose for which they were designed, but they lose the chief advantage of Belling's original technique, its extreme simplicity and speed.

The aceto-carmin technique can be greatly improved and the preparations made permanent by adding to the fixing solution certain inert substances which do not alter the fixation image but which serve as mounting media when the acetic acid and water evaporate. A number of such substances are available, *i.e.,* dextrose, gelatin, glycerine, gum-arabic, pectin, etc. A detailed investigation of these water-soluble mounting media is now in progress. Two mixtures, however, have already shown their usefulness.

(1) The specimen is macerated on a slide in a drop of Belling's aceto-carmin (a saturated solution of carmin in 45 per cent. acetic acid plus a trace of iron). Then several drops of the following solution are added:

[1] *Amer. Nat.,* 55: 533, 1921.
[2] *Biol. Bul.,* 50: 160, 1926.
[3] *Stain Tech.,* 4: 53, 1929.
[4] *Stain Tech.,* 6: 107, 1931.
[5] SCIENCE, 81: 75, 1935.
[6] *Amer. Nat.,* 70: 406, 1936.

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THE PLANT VACUOLE

CONWAY ZIRKLE

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The first vacuoles to be noticed by microscopists were not the large, seemingly empty regions in fully differentiated plant cells, but the smaller pulsating bodies which can easily be seen in many protozoa. These latter, which we call "contractile vacuoles," were described by Spallanzani as early as 1776 and, although he misinterpreted their function and assumed that they were respiratory organs, his description was remarkably precise and accurate. "It (the vacuolar organ) consists of two stars, with a very minute globe in the center, and situated, as one may say, in the foci of elliptical animalcula of the largest or middle size. Whether the animalcule moves or not, the stars are always in alternate or regular motion. Every three or four seconds the minute central globules swell like a bladder to three or four times their natural size and then fall; the inflation and eflation are performed very slowly. The same is done by the rays of the stars, except that inflation of the globe empties the rays, and inflation of the rays empties the globules." These organs were also described by many of the protozoologists who followed Spallanzani and they were the bodies to which the term "vacuole" was first applied (Dujardin, 1841).

The characteristic vacuole of the mature plant cell is so structureless and so passive that it failed to attract the attention of the eighteenth century biologists, and when it was finally noticed it was described merely as a part of the cell, distinct from the dense, granular cytoplasm whose streaming seemed so spectacular. Cytoplasmic streaming had been discovered by Corti (1774, 1776), and the discovery substantiated by Fontana (1776), but it was promptly forgotten until 1810 when it was rediscovered by Treviranus. Amici (1818, 1824), Agardh (1826), Meyen (1827, 1835, 1838), Slack (1834), Dutrochet (1837) and Schleiden (1842) investigated

the phenomenon in detail, and Meyen (1835, 1838), in his illustrations of the cytoplasmic stream, delineated the vacuoles very clearly. Schleiden (1842) recorded the physical differences between the streaming cytoplasm and the cell sap as follows: "In most plants in the families of the Characeae, Najadaceae and Hydrocharitaceae, there is observable in each cell a simple current ascending on one side and descending on the other, the fluid constituting which differs in color, consistence (mucosity) and insolubility in aqueous fluids, from the remainder of the transparent cell-juice." In the ends of certain desmid cells, (Naegeli (1849), Cohn (1854), DeBary (1858) and Fischer (1884) described the specialized vacuoles which contained crystals of gypsum and Charles Darwin (1875), and later Gardiner (1885) and deVries (1886), recorded the fragmentation of the colored vacuoles in the tentacles of *Drosera rotundifolia,* changes which accompanied the movements of the tentacles of this insectivorous plant. Went (1888) reached the general conclusion that all plant cells were vacuolate and Hof (1898) showed clearly that there were vacuoles even in the cells of root meristem, where the cytoplasm was densest.

The use which could be made of vacuoles in the investigation of osmosis seems to have been realized first by deVries (1877, 1884) who pointed out the fact that the cytoplasmic layer in the larger plant cells apparently acted as a perfectly semi-permeable membrane. The rôle of turgor in maintaining the shape and rigidity of plant tissue had long been known and Dutrochet (1828) had pointed out the connection between the osmotic pressure in the petioles of sensitive plants and their reaction to stimuli. Plasmolysis had been described by Pringsheim (1854), Naegeli (1855) and others, but it was not until the eighth decade of the last century that a thorough investigation of the osmotic properties of plant cells was undertaken (deVries, 1877, 1884; Hamburger, 1883; and others). Today vacuoles are of interest to biologists chiefly because of their importance in the investigation of osmosis and permeability. The enormous literature in this field has been summarized adequately by Jacobs (1924, 1935) and Osterhout (1931, 1936) and, accordingly, it need not be reviewed here.

Recently, an attempt has been made to homologize the plant vacuole with the Golgi-apparatus of the animal cell and a number of investigators have utilized the more modern cytological methods

in an effort to answer the questions which have been raised. Reviews of this cytological literature have been published by Dangeard (1923), Guilliermond (1929, 1930, 1933), Kechoski (1932) and Weier (1933) and the reader is referred to these papers for a more complete bibliography of the subject.

ORIGIN OF VACUOLES

The first investigators who described vacuoles were not particularly interested in their origin. There seems to have been a general though tacit assumption that they appeared *de novo* as the cytoplasm accumulated enough water to form visible droplets. Undifferentiated or meristematic cells were held to be non-vacuolate and the vacuoles were thought to develop as concomitants of cell enlargement and tissue differentiation. DeVries (1885), however, challenged this generally accepted view and argued that vacuoles were developed from distinct primordia which he called *tonoplasts.* These tonoplasts were supposedly small plastid-like bodies which occurred in meristematic cells and which multiplied only through the division of pre-existing tonoplasts. As they absorbed water they enlarged and became vacuoles, the tonoplast itself developing into the vacuolar membrane. Van Tieghem (1889) accepted deVries' conclusions but renamed the tonoplasts *hydroleucites.* Went (1888) did not endorse the view that vacuoles were derived from "massive" tonoplasts but held that they were derived from pre-existing vacuoles, and when he demonstrated that not only the reproductive cells of algae but also meristematic cells of the phanerogams were vacuolate he reached the broad general conclusion that "all plant cells contain vacuoles."

Unfortunately the methods used by deVries and Went in demonstrating the presence of vacuoles were open to criticism. Their technique for rendering vacuoles distinct was to place cells in a 10% to 15% solution of potassium nitrate. As Klebs (1890) emphatically stated, cells so treated were not normal. Furthermore, Went's demonstration of vacuoles in cells of marine algae by placing the plants in distilled water was unsound. For this reason his observations on living untreated cells were ignored and when Pfeffer (1890) experimentally caused vacuoles to arise *de novo* in the plasmodium of a Myxomycete by introducing into it soluble granules of asparagin, Went's conclusions were generally held to

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be untenable and the older view of Hofmeister prevailed, *i.e.*, that vacuoles appeared first in those cells which enlarged through the imbibition of water. Thus meristems are figured and described as non-vacuolate in most botanical text-books in spite of Hof's (1898) excellent demonstration to the contrary.

In 1910 Bensley found canals in the meristematic cells in the roots of *Allium, Lilium* and *Iris* which seemed to be identical with those found by Holmgren (1902) in liver cells and in epithelial cells of the suprarenal gland. Bensley showed that these canals were perfectly normal vacuoles which increased in size and ultimately fused to form the large central vacuoles of the fully differentiated plant cells. Other plant cytologists had missed seeing these canals because they had examined only those specimens which had been fixed in such a manner that all the cytoplasmic organization had been destroyed.

Recently, attention has been focussed again upon the origin and development of vacuoles by the work of Guilliermond (1914) and the Dangeards (1916, 1917, 1918, 1923). Guilliermond (1914, 1923, 1929, 1930, 1933) noted small colored vacuoles in the immature petals of a number of flowers. These vacuoles were of the size and shape of mitochondria and indeed were first looked upon as mitochondria by both Guilliermond and Dangeard, but the former, following Pensa (1917), demonstrated conclusively that they were very different from mitochondria in their chemical properties. These colored vacuoles were not highly specialized structures differing from the vacuoles in the primary meristem, but they were typical in every way except for their pigment. The emphasis placed upon them by Guilliermond and Dangeard was due to the fact that they were easier to see in living untreated cells than were the uncolored vacuoles in root tips and stem growing points.

Bailey (1930), working with living tissue, has shown that the initials of the cambium are vacuolated. Indeed, he reported certain cambial cells to be as highly vacuolated as plant hairs. He found a striking seasonal variation in the size, shape and number of vacuoles, the total volume of vacuolar material being greatest during the period of rapid cell division. In tissues produced from the secondary meristem, the vacuoles are obviously not derived from minute viscous mitochondria-like bodies. Actually, the vacuoles in the secondary meristem have no general form nor are they derived from any characteristic primordia. They may be rod-shaped, thread-like or spherical. They may fuse into a single large central vacuole or fragment into many separate globules which later may become beaded chains or tangled skeins. In many cells, the vacuoles are carried along in the streaming cytoplasm and the form they assume is conditioned by the cytoplasmic activity.

The vacuoles in the primary meristem are essentially like those in cambium (Zirkle 1932). All of the cells are vacuolate and the shape and size of the vacuoles seem to be conditioned primarily by the activity of the cytoplasm and the size of the cell. When the cytoplasm is quiescent, they tend to become spherical in the smaller cells; when it streams actively, they either become rod-like or are drawn out into Holmgren canals, or they may even form a reticular apparatus. Thus the shape of the vacuoles is of little importance in itself, for it merely indicates the activity of the cytoplasm. As a rule, the larger the meristematic cell, the greater the relative amount of vacuolar material, and in the exceptionally large apical cell in the root of *Osmunda* they may be as large as they are in the cambium of *Pinus* during the growing season.

There is no evidence that vacuoles in either the primary or secondary meristem develop from tonoplasts, hydroleucites, mitochondria or any other cytoplasmic inclusion and, while the smaller vacuoles may often assume the forms ascribed to these primordia, no useful purpose is served by using any of these terms. All meristematic cells which have been inspected adequately contain vacuoles (Hof, 1898) which are constantly changing their shape, fusing and again fragmenting. The only mode of origin that could be discovered for any individual vacuole was the division of a pre-existing vacuole, as reported by Went (1888). Guilliermond (1923) has stated that vacuoles originate *de novo* in *Saccharomyces* and *Saprolegnia*. The fact that this has not been observed in any meristematic cells does not mean, of course, that it never occurs there, for it is quite possible that a new vacuole could be mistaken for a fragment of a dividing one in a cell whose contents are in rapid motion. The fact that new vacuoles can be made artificially (Klebs 1890, Pfeffer 1890, Mollendorf 1936, *et al.*) has no real bearing on this problem as it does not justify the inference that such vacuoles occur in nature.

THE PLANT VACUOLE AND THE ANIMAL GOLGI APPARATUS

When Bensley (1910) found that the canals described by Holmgren in cells of liver and kidney epithelium occurred also in root meristems, he suggested that these canals represented the Golgi apparatus in both animals and plants. As it was a very simple matter to demonstrate that these Holmgren canals developed into the large central vacuole of the mature plant cell and, even, that they themselves were smaller vacuoles drawn out into a canalicular form by the streaming cytoplasm, he inferred that the plant vacuole was the homologue of the animal Golgi apparatus. Guilliermond and Mangenot (1922), Dangeard (1923), Parat and Painlevé (1924, 1925), Guilliermond (1927), Scott (1929), Bose (1931) and others have accumulated a great deal of evidence to support this view. On the other hand, Bowen (1926, 1927, 1928, 1929), Gatenby (1929) and Beams and King (1934, 1935) hold that vacuoles and the Golgi apparatus are separate structures and that the Golgi material in the plant cell constitutes the "osmophillic platelets," a distinct cell organ. Weier (1932, 1933), after studying Bowen's preparations, reported that the Golgi zone is similar in many respects to plastids.

In spite of their differences of opinion as to what constitutes the Golgi apparatus in plants, these investigators really agree in all of their essential findings. There is little argument as to questions of fact and little question but that some of their inferences are reared on an insufficient factual basis. As Bowen (1926, 1927) started the controversy, it would be well to summarize his conclusions briefly and to cite the evidence upon which they are based. He stated that he was entering the field of plant cytology without any particular bias as to the possibilities in prospect and that he would attempt to apply to plant cells those methods which had long been familiar to animal cytology. At the outset he discarded Golgi's original technique of *reducing* silver in a reticular apparatus on the ground that silver nitrate will *"impregnate"* almost anything on occasion. He relied primarily for his identification of the Golgi material upon its ability to reduce osmium tetroxide when the osmium was protected by such oxidizing agents as chromic acid or potassium bichromate, although he recognized that plant vacuoles which contained tannin would also reduce the osmium. Bowen concluded that there was a special Golgi material, that the reticulum

was but one of a number of forms that it assumed and that the material was of a fatty nature. Specific tests for fats generally failed to give positive results, but Bowen cited two instances where Golgi material contained fatty substances (Weiner 1926, Cowdry 1911). He concluded that "So far as fat tests have succeeded, they indicate the presence of Golgi substances as a material reality." The plant vacuole could not be composed of the Golgi material because the vacuole contents were "never lipoidal but always watery."[1] The real homologue of the Golgi apparatus in plants was the *osmophillic platelets*. Gatenby (1929) is in essential agreement with Bowen while Beams and King (1935), by means of the ultra-centrifuge, have shown that the osmophillic platelets in the onion root are distinct from the vacuole and that their relative specific gravity is comparable with that of the Golgi apparatus.

The alternate interpretation of the plant Golgi apparatus is based on a number of resemblances between the Golgi apparatus of animals and the plant vacuole both as to size and shape, and reaction to cytological reagents. Ten years before Golgi (1898) reduced silver in the reticular apparatus and thus discovered the structure which bears his name, Bokorny (1888) reduced silver in the vacuoles of *Spirogyra* from a very weak solution (1:100,000) of ammoniacal silver nitrate. Three years previous to this, deVries (1885) reduced osmium in plant vacuoles from a 1% solution of osmium tetroxide in 10% potassium nitrate. It is interesting to note here that the essentials of the two most usual methods for demonstrating the Golgi apparatus in animal cells were first used to "stain" the plant vacuole. Bokorny interpreted the reaction as indicating the presence of an active (reducing ?) protein in the cell sap. Klemm (1892) objected to this interpretation, however, and rightly pointed out that before the presence of this active protein could be demonstrated in the cell sap it would first be necessary to prove that the vacuole contained no tannin or other reducing agent. DeVries considered the reduction of osmium to be due to tannin. He also discovered that vacuoles were fixed with salts of other heavy metals, $HgCl_2$, $AgNO_3$, $CuSO_4$, etc. Loew and Bokorny (1877) showed

[1] Vacuoles are essentially aqueous although there is evidence that they contain a finely dispersed partially hydrated lipoid (Scarth, 1926). Bailey and Zirkle (1931) and Lison (1935) show how it is possible to explain the virage of vacuoles stained with vital dyes by assuming that they contain traces of a finely diffused fatty component.

that the content of the vacuoles was precipitated in a granular form by bases. The usual cytological preparations fixed by acids showed the vacuoles as hollow spaces.

It seems remarkable that Holmgren canals were not demonstrated in plant cells until 1910. The delay was undoubtedly due to the fact that plant meristems were investigated only in specimens fixed with reagents which, while preserving a high degree of nuclear detail, disorganized the cytoplasm and destroyed its finer structure. It was not until a different type of fixation was tried that an accurate picture of the cytoplasm and vacuoles was obtained and the resemblance between the vacuoles in plant meristems and the reticular apparatus of animal cells was demonstrated. These more modern fixing fluids depend for their characteristic images either upon formaldehyde or upon some bichromate in a solution on the basic side of pH 4.8–5.2. A fatty acid in the mixture destroys the image (Zirkle 1933). Thus Hof, Holmgren and Bensley were able to investigate accurately the size and shape of vacuoles but only as "negatives," for vacuoles, which contain no tannin, appear merely as vacant regions surrounded by cytoplasm. On the other hand, when tannin is present, the vacuole can be preserved and colored by the salts of any heavy metal. Iron is particularly useful, as Dangeard (1923) and Zirkle (1932) have pointed out.

Obviously, the identification of the Golgi apparatus in the plant cell is largely a matter of definition. Just what is meant by the Golgi apparatus? We really know very little about it; by comparison, our knowledge of vacuoles is extensive and precise. Vacuoles have long been recognized as perfectly normal parts of living plant cells. Their behavior in living tissue and reaction to vital stains have been described by a number of workers (Guilliermond, Dangeard, Irwin, Bailey, etc.); their contents in certain coenocytic forms have been analyzed by numerous investigators (Wodehouse 1917, Crozier 1919, Brooks 1922, Osterhout 1922, Hoagland and Davis 1923, Irwin 1923, etc.). On the other hand, the reticular apparatus discovered by Golgi has been definitely recognized only in tissue which has been subjected to prolonged and complicated chemical treatments (Cowdry 1924). Parat and Painlevé (1925) have indeed found a reticular structure in living animal cells which stains with Neutral Red and it is quite possible that this reticulum contains reducing substances which react with silver and osmium

to form the Golgi apparatus. Neutral Red, however, is no certain test for a vacuole, for it also stains certain cytoplasmic granules while it does not collect in vacuoles more basic than pH 6. The Golgi technique depends primarily upon the reduction within the cell of some heavy metal—generally silver or osmium. Chemically these tests are so nonspecific that it is impossible to make any precise inference as to the nature of the Golgi material. Any vacuole which contained a chloride—and most vacuoles contain chlorides—would retain the silver. A vacuole which contained tannin would be blackened by both silver and osmium. Osmium would be reduced also by oil droplets, oleoplasts, fatty acids, etc. Indeed, Ludford (1924) defined the Golgi apparatus as ". . . that region in the cytoplasm which brings about the reduction of osmium tetroxide at a lower temperature or in a shorter time than is required to produce a total blackening of the cell." As far as we know at present, the Golgi material or the osmophillic platelets may be composed of any one of a number of substances or a mixture of many substances. And the material that reacts to the Golgi technique in one cell may be very different from the material that reacts to the technique in another cell. We have no right at present to speak of a specific Golgi material. Golgi himself described a "reticular apparatus," but later work has shown that the material in this apparatus need not always have a reticular form. We should remember, moreover, that the only "reticular apparatus" found thus far in the plant cell is the vacuole drawn out by the streaming cytoplasm. It is obvious that before we may homologize the Golgi apparatus with any known structure in the plant cell, we will have to define the term much more precisely than we have thus far succeeded in doing.

CONTRACTILE VACUOLES

Both the regular pulsating vacuoles and those which discharge at irregular intervals are most easily observed in the protozoa and, consequently, their structural development and their function have been studied most extensively in this group. Indeed, the very existence of such vacuoles in plants has often been overlooked, although Lloyd (1928) has shown that they are to be found in the colored flagellate *Euglena,* in the zoospores of many algae and in the Myxomycetes. A very specialized type occurs in those vege-

tative cells of *Spirogyra* which are developing into gametes (Lloyd 1926, 1928). As the cells contract, previous to their passage into the conjugating tubes, special contractile vacuoles form and empty the cell-sap from the large central vacuole into the surrounding medium.

SALT CONTENTS OF VACUOLES

The contents of vacuoles can be analyzed directly only in such favorable material as the marine coenocytic alga, *Valonia,* and the aquatic group, the Characeae. *Valonia* is a spherical green alga composed of but a single multinucleate cell which often attains a diameter of 5 cm. The large central vacuole in the mature plant sometimes contains more than 25 cc. of cell sap, enough to be analyzed accurately. The cylindrical internodal cells of *Chara* and *Nitella* frequently attain a diameter of 1 mm. and sometimes reach an extreme length of 15 cm. The vacuolar contents of these cells can also be analyzed and the findings are exceptionally important, although we must be careful not to assume that all plant vacuoles contain what are found in these extremely specialized cells.

As early as 1847 Naegeli stated that the cell of *Valonia* was filled with water which, on tasting, seemed to be even saltier than sea water. Famintzin (1860) also described the vacuole as filled with salt water. Meyer (1891) made a careful analysis of the cell sap and compared it with sea water. He found the vacuoles to contain sodium, potassium, calcium and magnesium; chlorides, sulphates, nitrates and phosphates, and he was astonished to note that they contained much more potassium and much less sodium than sea water. More recent analyses have been made by Wodehouse (1917), Crozier (1919) and Osterhout (1922). The following table comparing the electrolytes in the cell sap of *Valonia* with Bermuda sea water is from Osterhout (1922):

Element	Sea water, parts per thousand	Cell sap, parts per thousand
Chlorine	19.6	21.2
Sodium	10.9	2.1
Potassium	.5	20.1
Calcium	.45	.7
Magnesium	1.31	trace
SO_4	3.3	.005

Brooks (1922), Irwin (1923) and Hoagland and Davis (1923) have analyzed the cell sap of *Nitella.* The following table is from the analysis of Hoagland and Davis:

Analysis of Nitella Sap and Pond Water

Element	Sap, parts per M.	Pond water, parts per M.	Factor of concentration
Sodium	230.	5.	46
Potassium	2120.	trace	?
Calcium	410.	31.	13
Magnesium	430.	41.	10
Chlorine	3220.	32.	100
SO_4	800.	31.	26
PO_4	350.	.4	870

A glance at these two tables shows that certain electrolytes penetrate the semi-permeable layer of cytoplasm and accumulate in the vacuoles *against an osmotic gradient* until they are far more concentrated within the cell than they are in the surrounding medium. Attempts to explain this behavior led to the present intensive investigation of permeability, one of the most complicated problems in all physiology. For a clear discussion of this problem the reader is referred to Jacobs (1924, 1935) and Osterhout (1928, 1936). It will be sufficient here to suggest two simple explanations. First, the penetrating substances may be combined chemically with something in the vacuole, forming a compound which cannot diffuse out from the cell; second, that some condition of the vacuole, such as its pH, alters the electrolytes from a relatively penetrating form to a relatively non-penetrating form. These explanations will be discussed briefly later.

Our knowledge of the contents of typical plant vacuoles which are too small to be analyzed directly is much more fragmentary. Undoubtedly, free oxalic acid exists in the vacuoles of many species, for Kerr (1933) has shown that it is in vacuoles of the root hairs of *Limnobium,* and Chester and Whittaker (1933) have found it in the expressed juice of a number of plants. Indeed, the higher plants can be divided into two groups: those whose expressed sap contains oxalic acid, and those whose expressed sap contains calcium chloride. When extracts from these two groups are brought together there is a white precipitate of calcium oxalate, often copious

enough to be mistaken for a precipitation reaction of the plant proteins (Chester and Whittaker).

SPECIFIC GRAVITY AND OSMOTIC VALUE OF VACUOLES

Mottier (1899) centrifuged cells from a number of different plants; from various algae, from leaves of mosses, *Elodea, Valisneria;* from the staminal hairs of *Tradescantia;* from trichomes of *Urtica, Cucurbita,* etc.; from the seedlings of *Zea, Vicia, Ricinus,* etc. He found that the colorless, fully hydrated vacuoles were lighter than the cytoplasm and were displaced toward the centripetal end of the cell. Milovidov (1930) substantiated the discovery of Mottier but found, in addition, that the smaller vacuoles of rose petals which contained anthocyanin were heavier than cytoplasm and were displaced centrifugally, as were the smaller vacuoles in the root tips of *Hordeum.* Beams and King (1935), by means of the air-driven ultra-centrifuge, obtained a force 400,000 times gravity and were thus able to place the various cell organs in separate layers. They found the vacuoles in the root tips of *Phaseolus* to be lighter than the cytoplasm and osmophillic platelets but heavier than the lipoid-like material and the fat globules. Incidentally, this seems to furnish the best proof to date that the osmophillic platelets are not composed of the simple fatty substance assumed by Bowen to be the Golgi material, as their specific gravity is greater than that of the fully hydrated vacuoles.

The osmotic value of the cell sap has been investigated by two different methods, both of which are inaccurate to some extent. The first, initiated by deVries (1884), consists of placing specimens in fluids of known osmotic strength and recording the osmotic value of the solution when incipient plasmolysis occurs. Such solutions are supposedly isotonic with the cell sap. Fitting (1916), Hanning (1912), Ilgin (1915), Ursprung and Blum (1916), Beck (1928), Weber (1929) and many others have used this method. Recently, Ernest (1935) has shown that this method does not yield exact results because of the time required for the plasmolysis to show. In fact, where the time lag is great, the plasmolysis may be due to injury. Approximate values may be obtained by this method, however, and she reports that the osmotic pressures of the cell sap in several species of *Iris, Saxifraga* and *Isatis* seem to

lie somewhere between that of a .4M and .5M solution of cane sugar or somewhere between 9 and 11 atmospheres.

The second method consists in pressing out the sap from the plant to be investigated and measuring its osmotic value directly. Such fluids, of course, contain cytoplasm, intercellular fluids, water from the xylem ducts, stored sugar, etc., and, consequently, their osmotic values may differ greatly from those of the vacuoles. The tremendous range in the osmotic value of the juices extracted from different plants, however, indicated that there is also a great variation in the osmotic values of different vacuoles, for it would be unreasonable to assume that there is no correlation whatever between the expressed juice and the vacuolar sap.

The osmotic value of the expressed juices of aquatics, as a rule, is much less than that of land plants. In *Elodea* it may be as low as 5 atmospheres. In the rain forests of Jamaica, Harris and Lawrence (1917) found it to range between 8 and 9 atmospheres in the herbs and between 11 and 12 atmospheres in the woody plants. In the leaves of mangrove trees, growing in salt-water swamps, it varied between 25 and 34 atmospheres. In certain plants from the Great Salt Lake region the expressed juice had the astounding osmotic value of 153 atmospheres (Harris, Gortner, *et al.,* 1921). Sen-Gupta (1935) has measured the osmotic value of the sap of a number of plants in India and finds that it ranges from 3 to 50 atmospheres. An excellent review of this subject is to be found in Miller (1931).

pH OF THE CELL SAP

Expressed plant juices are acid as a rule and are alkaline only rarely and then only slightly so. These juices, of course, are not composed merely of cell sap, although the acids found in them have frequently been located in the cell vacuoles. It is interesting to note that when tissue is crushed the juice which is first given off contains a much lower concentration of electrolytes than that obtained when greater pressure is applied so as to crush more of the cells. The reader is referred to the papers of Haas (1916, 1917), Atkins (1922), Smith and Quirk (1926). The following table is compiled from the data of Smith and Quirk. The fact is worth recording that the leaf of *Begonia lucerna,* which heads the table,

is the most acid tissue on record, for the juices range from pH .9 to pH 1.36, approximately as acid as .1 N sulphuric acid:

Plant	pH of Juice Expressed from Leaves
Begonia lucerna	0.9 – 1.36
Oxalis sp.	1.70 – 1.72
Rumex crispus	3.53 – 3.56
" *obtusifolia*	3.64 – 3.67
Agave sp.	4.30 – 4.31
Allium cepa	4.33 – 4.45
Ananas sp.	4.72 – 4.74
Musa sp.	4.74 – 5.04
Nephrolepis exaltata	5.20 – 5.22
Polystichum lonchitis	5.32 – 5.35
Colocasia antiquorum	5.33 –
Tradescantia zebrina	5.44 – 5.47

The chief acid involved is oxalic.

The pH of the cell sap can be measured directly only with great difficulty and the values obtained are generally somewhat inaccurate. The acidity of the food vacuoles of certain protozoa has also been measured (Shapiro 1927, Howland 1928), together with the changes in pH which accompany the digestion of their inclusions. From a point approximating neutral (pH 6.6 – 7) they become as acid as pH 4.3 during digestion and then become more basic (pH 5.4 – 5.6) as digestion is completed. Particularly favorable plant material, where the pH of the vacuoles may be determined readily, is to be found in flower petals, colored berries, red cabbage leaves, etc., where the cells contain anthocyanin pigments dissolved in the cell sap. The color of these pigments varies with changes of acidity and, if the pigment is known, it is possible to obtain the pH of any solution in which it is dissolved by comparing the solution with a color standard. Indeed, these pigments were used as indicators of acidity by Boyle as early as 1664 when he employed an extract from violets. Wray (1670), Lister (1671) and Grew (1682) also described color changes which occurred when various plant pigments or flower petals were subjected to acids and bases, and the practical value of these pigments as indicators was soon established by the chemists. Schwarz (1892) correlated the change from red to blue during the anthesis of flowers of *Pulmonaria, Anchusa* and *Lathyrus* with a decrease of acidity,

and Willstätter (1914) noted that the same pigment was responsible for the color of both the rose and the cornflower, and assigned a value of pH 5.5 to the cell sap of the former and of pH 7.2 to the latter. Haas (1916) investigated the pH of a number of plant cells by means of these natural indicators. He extracted the indicators and recorded their color in buffers of known hydrogen ion concentration. By matching the buffered solution of these anthocyanin pigments with the pigments in the vacuoles of the living cells, he was able to obtain the pH of the cell sap. He found that the vacuoles ranged from pH 3 to pH 7 or even 8, depending upon the species investigated. Irwin (1919) and Brooks (1926) used these anthocyanin pigments to measure changes in the acidity of cell sap which occurred during physiological experiments. Buxton and Darbishire (1929) and Smith (1933) showed that there were a great many different anthocyanins, each with a characteristic color range. Smith recorded the pH of the cell sap in the petals of 25 species of flowering plants and found that it ranged from pH 3.1 to pH 7.8.

The hydrogen ion concentration in vacuoles which contain no natural indicators is more difficult to estimate. The vacuolar contents of *Valonia* and *Nitella* can be extracted in quantities sufficient to measure although when we consider the difficulty in maintaining the CO_2 tension we would expect a relatively large experimental error. Indeed, Crozier (1919) found the cell sap of *Valonia* to range from pH 5 to pH 6.7 in different specimens, the average being about pH 6. On the other hand, Hoagland and Davis (1923) reported that the cell sap of *Nitella* remained nearly constant at pH 5.2, even when the surrounding medium varied from pH 5 to 9. The pH value of the smaller microscopic vacuoles of course cannot be obtained by this method.

The fact that a number of basic dyes, which are pH indicators, will penetrate the cytoplasmic layer and accumulate in the vacuoles has led a number of investigators to estimate the pH of the vacuoles by their virage when stained. There are many sources of error in this method as the dyes in question have relatively large salt and protein errors and, in addition, an error called to our attention by Dangeard (1916) caused by a substance in the vacuoles named "metachromatin." Different vacuoles, sometimes within the same cell, may acquire very different colors when stained by such dyes

as Neutral Red (Dangeard 1916, Bailey and Zirkle 1931). Living cells in sections cut through cambium, phloem and medullary rays of a number of gymnosperms contain two categories of vacuoles (Bailey and Zirkle 1931), designated "A" type and "B" type for convenience. The "A" type vacuole contained tannin and was stained magenta with Neutral Red, the "B" type contained no tannin and was stained orange. The tannin-containing vacuoles were stained with 36 different basic dyes and their virage indicated that their hydrogen ion concentration was very close to pH 4.4. The "metachromatic" error did not enter into this measurement.

On the other hand, the range indicator method gave very uncertain results with the "B" type vacuoles. Twelve dyes were found which stained these vacuoles, and the color of the stained vacuole matched different buffered solutions of the dyes ranging from pH 7.2, when they were stained with Neutral Red, to pH 13, when they were stained with Brilliant Cresyl Blue, depending upon the particular dye that was used. Obviously, such results show that the vacuoles contain some substance which markedly alters the normal virage of basic dyes. If chloroform is shaken with the various buffered solutions, the dyes will be partitioned between the chloroform and the buffer when the two layers separate. When the buffer is as acid as pH 4.4, the apparent pH of the "A" type vacuole, the color of the dye is always the same in both layers. With more alkaline solutions, however, the color of the dye in the chloroform layer may be very different from its color in the buffer itself. With all of the twelve dyes employed, the color of the stained "B" type vacuole matched the color of the dye in the chloroform which was in contact with the solution buffered at pH 5.8. These results can be explained if we assume that the "B" type vacuoles contain some substance which alter the virage of the dyes in a manner comparable to the alteration caused by chloroform and that the pH of the vacuoles is approximately 5.8. This point will be discussed at greater length under the heading "metachromatin." Lison (1935) has also called attention to the fact that the virage of the basic dyes which penetrate the cytoplasmic layer is conditioned by this "metachromatic" factor and that the previous records of alkaline vacuoles based upon the virage of these dyes are in error.

The sulphonated dyes used as indicators by Clark and Lubs are insoluble in chloroform and ether and are not subject to the "metachromatic" error. These dyes, however, do not penetrate the cytoplasmic layer as do the basic dyes, but they can be inserted into the vacuoles directly by the modern technique of micro-injection and once in the vacuoles they are unable to diffuse outward. Chambers and Kerr (1932) have injected them into the living root hairs of *Limnobium,* and find that the vacuoles there have a pH of 5.2 ± .2.

METACHROMATIN AND THE COLLOIDAL CONTENTS OF VACUOLES

The idea that vacuoles contain metachromatin can be traced to the early conception that they originated from the hydration of relatively dense colloidal globules. These globules were labelled metachromatin because of their reaction to vital dyes. Like bodies had been reported in bacteria (Babes 1889, 1895) and in yeast (Guilliermond 1902). In the latter the globules fused to form a larger more hydrated body which had the property of concentrating the usual vacuolar stains. The investigations of both Guilliermond and the Dangeards showed that the vacuoles of the higher plants contained some substance which affected their color when they were stained with vital dyes and which ultimately united with the dye to form precipitates. The existence of some such substance has been amply confirmed by the subsequent work of Mangenot (1929), Dufrenoy (1929), Bailey and Zirkle (1931), Lison (1935) and others.

The colloidal nature of certain highly specialized vacuoles in storage tissue was recognized as soon as it was shown that they developed into aleurone grains. Aleurone grains had been seen and described by Hartig (1855, 1856), Halle (1858) and Maschke (1859), while Pfeffer (1872) showed that they were composed essentially of proteins. It was first thought, however, that these grains were developed from a form of plastid that occurred in such tissue as the endosperm of *Ricinus* and in a number of other seeds. Maschke located the grains in vacuoles and Gris (1864) and Wakker (1888) showed that the grains themselves were partially dehydrated vacuoles. Guilliermond (1907) and Dangeard (1923) especially have traced the cytologic development of these grains in great detail, and the evidence seems conclusive that reserve protein is stored in vacuoles which, consequently, are colloidal.

There is also good evidence that other types of vacuoles contain colloids. Perhaps all vacuoles are more or less colloidal although many contain but slight traces of organic compounds. Bokorny (1888) held that vacuoles contained protein, but on very insufficient evidence. Weber (1925) and Frey (1926) assumed the presence of colloids to explain the observed viscosity of the vacuolar contents, the latter measuring the rate at which the gypsum crystals settled downward in the end vacuoles of desmids. Bailey (1930) described the formation of Liesegang rings when certain "B" type vacuoles were stained with Neutral Red and, as these vacuoles contained no flavones or other such substance, the presumption is that the included colloid may be of a type that is very widely distributed in the vegetable realm. Indeed, many of our observations would be explained very simply if we assume that "metachromatin" itself consists of finely dispersed colloidal particles of some fatty substance. Its fatty composition would explain its effect upon the virage of vital stains as its characteristic color effects have been shown to resemble those of chloroform (Bailey and Zirkle 1931) and of ether (Lison 1935). Such a substance evenly dispersed in an aqueous medium would probably be in colloidal particles. Scarth (1926) has shown that the colloidal contents of certain vacuoles behave as partially hydrated lipoids.

TANNIN

Tannin was definitely identified in cell sap by Wigand (1862) and Wiesner (1862) when they treated the cells with iron salts and with alkalies. With the former the sap turned blue, green or black depending upon the species tested and the particular part of the plant from which the specimen was taken; with the latter the sap became orange or yellow. The tannins were first investigated by a number of French and English chemists between 1790 and 1800, and the subsequent publications on the subject are far too numerous to be cited in a review of vacuoles, although many of the studies of tannin are cytological. The reader is referred only to some of the more recent work, that of Wisseling (1910, 1914), Lloyd (1922), Guilliermond and Dangeard in numerous papers and of Mangenot (1927, 1929).

That tannin is widely distributed throughout the vegetable kingdom was shown by Bailey and Zirkle (1931) who report its occurrence in 272 representatives of 42 orders and 90 families of the Pteridophyta, Gymnospermae and Angiospermae. It is found especially in the phloem, in the mesophyll of leaves, in fruit and insect galls, mixed with pyrogallol, and in the peripheral regions of young roots. Sometimes the vacuoles contain only traces of tannin, as in certain cells in the root tips and young leaves of *Pinus*. When such tissue is fixed with fluids containing heavy metals, precipitates form about the rims of the smaller vacuoles and they appeared as hollow spheres, circles or platelets, with "osmophillic" peripheries and "osmophobic" centers.

TONOPLASTS

The membrane which surrounds a vacuole is called a tonoplast and although cytologists are not in agreement as to its structure or composition or even as to whether it is part of the vacuole itself or of the cytoplasm, there is little doubt but that the vacuole is surrounded by a layer which is impervious to many of its components. The name tonoplast was introduced, as has been stated, by deVries (1884) who looked upon it as a plastid-like vesicle which grew by hydration into a vacuole. Van Tieghem (1888), who accepted the tonoplast theory temporarily, and Went (1888) collected much evidence in favor of deVries' hypothesis but most botanists discarded it, so today the word tonoplast is merely the name given the membrane which surrounds the vacuole and the use of the term does not imply the acceptance of any theory of vacuole origin.

Recently, the tonoplast has been investigated by a number of physiologists. Lloyd and Scarth (1926) have shown that it has many of the physical properties of lecithin and that some of its characteristic activities are not dependent upon the life of the cell. Indeed, many investigators have isolated tonoplasts with their enclosed vacuoles, and find that they maintain many of their typical characters even when floating free from the cell (Bailey 1930, Chambers and Hofler 1931, Plowe 1931, Eichberger 1934, Lederer 1935). On the other hand, tonoplasts are undoubtedly a part of the cytoplasm in living plant cells as they can be observed to flow as a part of the cytoplasmic stream. Indeed, Weber even considers the evidence of an inner membrane of the cell as incomplete (See Weber 1932, Hofler 1932). Obviously, there is no need to con-

sider the tonoplast as a definite membrane differing in chemical composition from the rest of the cytoplasm. Its very contact with the cell sap would give it properties not possessed by the remaining cytoplasm. Whatever its origin or composition, it acts as a membrane impervious to many substances, and it thus resembles the plasma membrane, although it differs in certain respects for Osterhout, Damon and Jacques (1927) have shown that the inner and outer surfaces of cells differ.

Micromanipulative investigations have shown that the vacuolar membrane is tough, elastic and sticky (Chambers and Hofler 1931, Plowe 1931, Eichberger and Lederer 1934). Mothes (1933) holds that it is extremely thin, homogeneous, elastic and non-miscible with water or cytoplasm. It contains an essential albuminous component, although he found no evidence of a lipoid constituent. The permeability of the tonoplast will be discussed later.

THE VITAL STAINING OF VACUOLES

Strictly speaking, it is perhaps inaccurate to describe vacuoles as ever being really stained. They are certainly not stained in the sense that chromosomes, cell walls or textiles are stained. They merely become colored through their ability to concentrate weak solutions of basic dyes, just as oil droplets concentrate Sudan IV from dilute alcoholic solutions. A "stained" vacuole is thus essentially a colored solution, although frequently the basic dye which is absorbed unites chemically with certain of the acids dissolved in the cell sap. This ability of vacuoles to absorb and accumulate dyes and other electrolytes against an osmotic gradient is one of their most interesting properties and it has stimulated some of the most fundamental physiological research in recent years.

As has been stated, two explanations suggest themselves. The first is that the cell-penetrating electrolytes combine with something in the vacuole to form a non-diffusible compound. The electrolytes would then be trapped within the vacuole until all of the hypothetical components were used up, when, of course, equilibrium would be reached and no more dye would accumulate. Scarth (1926) reports that in certain cases the penetrating dyes combine with colloidal material in the cell sap. This first explanation certainly accounts for the observed behavior of the "A" type or tannin-containing vacuoles. Basic dyes which penetrate the

cytoplasmic layer color these vacuoles brilliantly, forming precipitates as staining progresses—often until the vacuoles are nearly filled—whereupon the remaining cell sap loses its color (Bailey 1930, Bailey and Zirkle 1931). This explanation is inadequate, however, when applied to the "B" type vacuoles, which contain no tannin, although perhaps basic dyes combine with some vacuolar substances in all vital staining.

Before proceeding with the discussion it will be well to emphasize a factor in the problem of permeability which has generally been overlooked, and, in consequence, a number of unnecessary errors have appeared in many of the earlier contributions. We must remember that before a dilute solution of a dye can "stain" a vacuole, it must both penetrate the cytoplasmic layer and accumulate in the vacuole itself. These two processes occur at the same rate, of course, only when none of the penetrating dye diffuses out from the cell. If the dye leaves the cell as rapidly as it enters, the vacuole will not be colored, for a solution of the dye in the vacuole as dilute as it is in the exterior solution will show no color upon microscopic examination. Thus the failure of a dye to stain a vacuole is no evidence of the dye's inability to penetrate the cytoplasmic layer. Much of the criticism of Overton's classical hypothesis, that substances soluble in both water and fats penetrate the cell with relative rapidity, has been based on the alleged inability of certain fat-soluble dyes to enter the cell. Thus Rhodamine (vital) has been cited as a lipoid-soluble dye which penetrates living cells extremely slowly when it really accumulates in vacuoles, which can trap it with tannic acid, as rapidly as any other dye that has been investigated (Bailey and Zirkle 1931). Likewise, the cells of *Nitella* have been reported as impervious to Methyl Red regardless of the pH at which staining was attempted. This dye does not accumulate in "B" type vacuoles (the type of *Nitella* vacuole) but it penetrates the cytoplasmic layer very rapidly and accumulates in "A" type vacuoles. Kerr reports that it does not stain the vacuole in the root hair of *Limnobium* (a "B" type vacuole) but that it accumulates very rapidly in such vacuoles when tannic acid is injected into them. In spite of Osterhout's warning (1916), the neglect of this very real distinction between rates of penetration and rates of accumulation has led to a somewhat oversimplified picture of the effects of pH upon the rates of penetration.

Apparently any basic dye which can penetrate the cytoplasmic layer will be trapped by a vacuole which contains tannic acid. Bailey and Zirkle (1931) found 35 basic dyes which stained the "A" type vacuole, but only 12 of these dyes accumulated in the "B" type, although they penetrated the cytoplasmic layer. On the other hand, acid dyes, which are insoluble in lipoids, apparently cannot enter the living plant cell, for no such dye was found to be permeable out of the 36 dyes tested. When such non-permeating dyes are injected into vacuoles (Chambers and Kerr 1932) they do not diffuse outward, as do the lipoid-soluble basic dyes, but remain in the cell until death occurs. They can neither get into nor out of living cells.

Each of the permeable dyes has a characteristic pH range at which it can enter the cell (Bailey and Zirkle 1931). Such dyes as Neutral Red, Brilliant Cresyl Blue, Methylene Blue, Auranin, Pyronin, etc., penetrate rapidly from basic solutions, slower from slightly acid solutions and not at all from buffers in the more acid ranges. On the other hand, Methyl Red, Ethyl Red, Brilliant Green, etc., penetrate more rapidly from acid solutions, slowly if at all from the more alkaline buffers. Rhodamine (vital) accumulates in the vacuoles at a uniform rate throughout the entire pH range at which the dyes were tested. Obviously, the assumption that dyes penetrate in the form of free bases will not explain all of the effects of the pH upon the rate of penetration of the dyes.

The second explanation of the accumulation of electrolytes in vacuoles assumes that some condition of the cell sap, such as its pH, alters the penetrating dye to a form which is less permeable. Such a dye would thereupon diffuse outward from the vacuole more slowly than it entered until it reached equilibrium at a relatively high concentration. Irwin (1923, 1925, 1926) measured very carefully the effects of pH upon the rates at which Brilliant Cresyl Blue accumulated in the vacuole of *Nitella*. She found that the dye accumulated rapidly from basic buffers, slower from neutral or slightly acid solutions, and not at all from solutions buffered at the pH of the cell sap. Thus the dye seemed to be trapped by the hydrogen-ion in the vacuole for from such solutions it could not enter the cytoplasmic layer. In agreement with this interpretation is the work of Bailey and Zirkle (1931) who reported that all of the 12 dyes, which they found to accumulate in the "B"

type vacuole, were dyes which entered the cell relatively rapidly from basic solutions but very slowly if at all from solutions at the pH of the vacuole itself. All of the 23 dyes which entered the cell but which did not accumulate in the "B" type vacuole penetrated rapidly from solutions buffered at the pH of this type of vacuole and thus they could not be held in the vacuole by a pH trap but could easily penetrate the cytoplasmic layer from the cell sap. It is very important for us to realize that electrolytes which accumulate in plant vacuoles are not necessarily those which penetrate more rapidly than those which do not accumulate there. As far as we know at present, those which accumulate may really penetrate more slowly and over a more restricted pH range. Further investigation will have to decide this question.

SUMMARY

Spallanzani described contractile vacuoles in animals as early as 1776. Meyer (1835) depicted the large central vacuole characteristic of the mature plant cell in his illustration of cytoplasmic streaming and Schleiden (1842) recorded the difference between the vacuolar contents and the cytoplasm itself. Charles Darwin (1875) first noted the fragmentation of the colored vacuoles which accompanied the movement of the tentacles of *Drosera,* and deVries (1877) called attention to the importance of vacuoles in the investigation of osmosis. Went (1888) reached the general conclusion that all plant cells contained vacuoles and Hof (1898) showed that even meristematic cells were vacuolate. Today vacuoles are of interest to biologists chiefly because of their importance in the study of permeability.

Plant vacuoles are derived from the division of pre-existing vacuoles. There is no definite proof at present, however, that they never originate *de novo*. Their size and shape in meristematic cells are determined primarily by the cytoplasmic activity. They may be either globular, rod-like, canalicular or tericulate. They show many resemblances to the animal Golgi apparatus, both as to their structure and their reaction to cytological reagents.

Vacuoles have the property of accumulating electrolytes against an osmotic gradient, both the inorganic salts necessary for plant nutrition and the basic dyes used for vital staining. Vacuoles, in general, have a specific gravity less than that of cytoplasm but

greater than that of the oil droplets in the cells. Their osmotic value in different species varies from about 3 to 150 atmospheres and their hydrogen ion concentration from pH .9 to pH 8.

In the endosperm of a number of seeds the vacuoles develop into aleurone grains. They contain much ergastic material. There is evidence that all vacuoles contain some colloidal material. Their staining reactions can best be explained by assuming that they contain some substance, metachromatin, that acts as a partially hydrated lipoid. Many vacuoles contain tannin, flavones or anthocyanin pigments. Oxalic acid is also found in the vacuoles of a number of different plants.

The vacuolar membrane is called a tonoplast. It is a liquid membrane in many cells and circulates with the cytoplasmic stream. This membrane is semi-permeable, like the plasma membrane. Any investigation of the accumulation of substances within the vacuole must be concerned with their inability to penetrate the tonoplast from the vacuole as well as their ability to penetrate the cytoplasmic layer.

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